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Formulation and Evaluation of Oral Mucoadhesive Microspheres of Levofloxacin Hemihydrate

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HUMAN

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Keywords: Levofloxacin Hemihydrate, Chitosan; Mucoadhesive Microspheres; Inotropic Gelation Technique.

ABSTRACT

The current study's goal was to create gastro-retentive microspheres for the controlled release of the medication to treat *H. pylori* infections more effectively by releasing the medication specifically in the stomach for a lengthy period of time utilising chitosan as a polymer. Ionotropic gelation was used to create floating mucoadhesive microspheres out of sodium alginate and chitosan. All of the formulations were evaluated using a variety of physicochemical criteria, and they were all found to be within acceptable bounds. In contrast, according to in vitro drug release assays, formulation F5 had a regulated release rate of 99.42% and the highest percentage of drug release. Consequently, since the zero order model's plots were linear, it might succeed in controlling drug release. In conclusion, extended GIT retention duration, decreased dose frequency, and increased levofloxacin bioavailability from the produced floating microspheres may all help in the delivery of anti-bacterial agents.

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1. INTRODUCTION

H. Pylori infection is most common amongst the world population which leads to stomach illness. The prevalence of the frequency of *H. pylori* infection has shown to be 44.3% worldwide due to lack of socioeconomic status, urbanization [1], and sanitary conditions. The infection spreads to people group via the oral-oral or fecal-oral route among family members. The path physiology of infection includes the infection to stomach initially in the lumen and then *H. pylori* settles in particular areas like the corpus and the antrum, where it can thrive in acidic environments and cause persistent infection. Following infection, a number of gastro-duodenal consequences, including gastritis, gastric ulcers [2], duodenal ulcers, symptoms of dyspepsia, gastric cancer, and gastric mucosa-associated lymphoid tissue (MALT) B-cell lymphoma, may manifest. This is serious issue and is considered [3] as the third most prevalent reason for cancer linked death is gastric cancer, which is still a serious public health concern. It caused the deaths of approximately 723,100 people in 2012. *H. Pylori* infection has recently been reported to induce a number of extra-gastric problems in addition to its link to gastro-duodenal issues. Diagnostic tests for *H. pylori* infection can be divided into two categories, invasive along with non-invasive methods. Invasive tests [4] involve an endoscopy of Upper GIT with gastric mucosal biopsy and either rapid urease testing, histology, culture or polymerase chain reaction (PCR) tests. The non-invasive tests include antibody detection, carbon labelled urea breath tests and stool antigen detection. A recent biopsy study confirms that after acquiring *H. pylori* penetrates [5] into the mucus layer of the stomach and fixes itself with glycolipids and phospholipids of mucus gel. *H. pylori*, then disrupts epithelial layer directly or indirectly by releasing of certain toxins and enzymes. For effective *H. pylori* eradication, antibiotics need to enter into the gastric mucus [6] layer and maintain an effective concentration for sufficient period of time. Drugs released from conventional tablets or capsules reside shorter duration of time in stomach. Because of its shorter residence time, conventional tablets and capsules are unable to deliver the antibiotics into [7] the mucous layer for sufficient period of time. The main causes of *H. pylori* eradication therapy's failure is this. In order to increase the eradication rate, it is essential to design suitable dosage forms to deliver the antibiotics into the site of infection. Non compliance, bacterial resistance, cost of drugs and duration of the treatment also influences the *H. pylori* eradication [8]. Antibiotic resistant *H. pylori* strains developed mostly due to the unavailability of required antibiotic concentration at the site of action for sufficient period of time. It could be a significant issue with *H. pylori*

treatment. It is essential to design suitable drug delivery systems to deliver the antibiotics into the mucus layer where *H. pylori* exist. GRT time transit of the dosage forms is important for delivery of drug into the mucus. Gastro retentive systems are commonly used to increase GRT [10] time of dosage forms. Mucoadhesive dosage forms adhere into the mucus layer and release the drug at a controlled rate with principles of bioadhesion and mucoadhesion [11-15] providing advantage of reduced dose frequency, prolonged release and release at the GIT site [9]. The levofloxacin hemihydrates [15-17] is a Quinolone (Dose 500mg) with anti-Bacterial and Anti-Infective property widely used in *H. Pylori* infection. The Mechanism of Action LFX involves destroying of bacterial to pro isomerase and di-nucleotide adenosine gyrase enzymes required for di nucleotide adenosine replication thereby transcription, and repair in the recombination. LFX exhibits *in vitro* MIC nearly 2 mcg/mL or less against most (90%) strains **(***H. pylori* infection). The aim of the present study is to create and develop mucoadhesive microspheres of levofloxacin hemihydrate for the treatment of *H. pylori* infection utilizing mucoadhesive polymers in order to minimize the dosing frequency reduce dose and also increase patient compliance, develop an optimized dose form.

MATERIALS AND METHODS

All the materials used for the study were of laboratory analytical grade. The drug levofloxacin hemihydrates were obtained as gift sample from Hetero labs.

Methods

PREFORMULATION STUDIES

Standard curve of Levofloxacin hemihydrate in 0.1 N HCl: To determine the inter- and intra-day fluctuations, a stock solution of Levofloxacin hemihydrate (100 g/ml) in 0.1 N HCl was made three times, each time in duplicate. To obtain the established [15] standard solutions in the range of 1–10 g/ml, it was further diluted. At 293 nm, absorbance was determined spectrophotometrically using a Shimadzu UV/Visible spectrophotometer 2100 from Tokyo, Japan. The calibration curve was created using the average data (n=9). The regression equation produced from the calibration curve was used to determine the drug's concentration in the dissolved state.

Preparation of microspheres: Levofloxacin hemihydrate microspheres of chitosan (CS) and sodium alginate (ALG) were created by ionotropic gelation method. By vigorously [18-20] swirling for 10 minutes, a weighed amount of micronized levofloxacin hemihydrate powder was extensively dissolved in the ALG solutions (1-3% w/v) in deionized water that contained 0.075% w/v sodium dioctyl sulphosuccinate (DOSS) as a surfactant. The calcium chloride solution (2.0% - 4%w/v) containing chitosan (CS) (1.0% w/v), which had previously been dissolved in acetic acid solution (0.5% v/v) (pH adjusted to 5.0), was immediately sprayed with the ALG- levofloxacin hemihydrate mixture. The final dry mass of the microspheres was measured after they were washed twice with distilled water and dried overnight at 37°C in an oven.

Formulation of the microspheres: [19] The 3*3 Factorial Design could result in L1-L9 Formulations. Increasing calcium chloride concentration from 2-4% varying concentrations of sodium alginate with 1% chitosan resulted in levofloxacin Hemihydrate (2.5% microspheres).

Table No. 1: Formulation of Levofloxacin hemihydrate microspheres (factorial design 3*3)

Alginate chloride				Levofloxacin hemihydrate
code	**(% w/v)**	**(% w/v)**	**(% w/v)**	**(% w/v)**
LCAM1	1	1	2	2.5
LCAM 2	2	1	2	2.5
LCAM 3	3	1	2	2.5
LCAM 4	1	1	3	2.5
LCAM 5	2	1	3	2.5
LCAM6	3	1	3	2.5
LCAM 7	1	1	4	2.5
LCAM 8	2	1	4	2.5
LCAM 9	3	1	4	2.5

Evaluation of Mucoadhesive Microspheres: Determination of percentage yield of Microspheres. The Prepared [22] microspheres were collected and weighed accurately using a

digital balance. The percentage yield of prepared microspheres was calculated by using the formula mentioned below:

$$\text{Percentage Yield of microspheres} = \frac{\text{Weight of microspheres of obtained}}{\text{Total weight of drug and polymers}} \times 100$$

- Determination of drug content and encapsulation efficiency: The drug content of the microspheres [20] were measured by extraction method. Accurately weighed 5 mg of mucoadhesive microspheres were crushed in to a powder using mortar and pestle. The crushed microspheres were placed in 100 ml of 0.1 N HCl (pH 1.2) and stirred for 2 hours using magnetic stirrer (100 rpm) at 37 ± 0.5^{o}C. The samples were then filtered to obtained clear solution and analyzed for the drug content.

$$\text{Drug content in microspheres} = \frac{\text{Weight of drug in microspheres}}{\text{Weight of microspheres}} \times 100$$

$$\text{Encapsulation efficiency} = \frac{\text{Actual drug encapsulated}}{\text{Theoretical drug encapsulated}} \times 100$$

Particle size analysis: Particle size of the drug, excipients and prepared microspheres were measured by using laser based particle size analyzer (780 AccuSizer, Particle sizing systems Inc, USA). The particles were dispersed inn-Hexane, and suspended mechanically by magnetic stirring during the analysis.

Shape and surface characterization: The shape and surface characteristics of the microspheres were observed under a Scanning Electron Microscope (Sem). Hitachi-Sem Model S – 450 model scanning electron microscope was used for the study. The prepared microspheres were placed directly on to the SEM sample holder by using double-sided fixing tape and coated with gold film (thickness 200 nm) under reduced pressure (0.001 torr) and photographed.

***In vitro* evaluation of mucoadhesiveness:** A periodic acid/Schiff (PAS) colorimetric method reported by Mantle and Allen[166] was used to determine the free mucin concentration in order to assess the amount of mucin adsorbed on the Levofloxacin hemihydrate

mucoadhesive microspheres and its effect [21-24] on the assessment of mucoadhesive behavior of prepared mucoadhesive microspheres. Two reagents were prepared. Schiff reagent contained 100 ml of 1% basic fuchsin (pararosaniline) aqueous solution and 20 ml of 1 M HCL. Sodium metabisulphite (0.1 g) was added to every 6 ml of Schiff reagent before use, and the resultant solution was incubated at 37°C until it became colorless or pale yellow. Periodic acid reagent was freshly prepared by adding 10 µl of 50% periodic acid solution to 7ml of 7% (vol/vol) acetic acid solution. Standard calibration curve were prepared from 2 ml of mucin standard solutions (0.25, 0.5, 0.75, and 1 mg/2 ml). After adding 0.2 ml of periodic acid reagent, the samples were incubated at 37°C for 2 hours in a water bath. Then, 0.2 ml of Schiff reagent was added at room temperature. Thirty minutes later, the absorbance of the solution was recorded at 555 nm in calibration a UV spectrophotometer (Spectronic 20D). Triplicate samples were run. All the samples were determined with the same procedure. The mucin content was calculated from the standard calibration curve. As comparison, the mucoadhesive potential of microspheres was also assessed with the above procedure. Each experiment was performed 3 times and standard deviation noted.

Adsorption of Mucin on Chitosan Microspheres: Mucin aqueous solution with different concentrations (0.025, 0.05, 0.1, 0.2, and 0.5 mg/ml) were prepared. Levofloxacin hemihydrates Mucoadhesive microspheres (20 mg) were dispersed in the above mucin solutions, vortexed, and shaken at room temperature. Then, the dispersions were centrifuged at 4000 rpm for 2 minutes, and the supernatant was used for the measurement of the free mucin content. The data obtained were interpreted using Freundlich or Langmuir equations describing the adsorption isotherms [23].

$$C_{ads} = KC_e^n$$

$$C_{ads} = \frac{aC_e}{b + C_e}$$

Where Cads is the concentration of mucin adsorbed at equilibrium and Ce is the concentration of free mucin at equilibrium. Values of different constants were obtained from the graphs of the above equations. For the Langmuir equation, 1/Cads was plotted against 1/Cfree to get the constants and for the Freundlich equation, log Cads was plotted against Cfree to get the constants. The mucin adsorption is estimated using the Equation.

$$\text{Mucin adsorption (\%)} = \frac{\text{Total mass of mucin} - \text{free mucin}}{\text{Total mass of mucin}} \times 100\%$$

Compatibility studies

Fourier-Transform Infrared Spectrophotometry (FTIR): Infrared red spectra for pure Levofloxacin hemihydrate, polymers, blank microspheres were obtained on a FTIR-[Shimadzu (84005)] spectrophotometer using the potassium bromate disk method. 200mg potassium bromate was used for the analysis of 2mg of Sample.

Differential Scanning Calorimeter (DSC): The thermal analysis of pure drug, formulations and blank microspheres were carried out using DSC Universal V4.2E TA instruments, to evaluate possible drug-polymer interaction. 3mg of sample was accurately weighed and placed in a 40µl aluminum pan and sealed with a punched lid. A temperature range of 10–300^{o}C was scanned using a heating rate of 10^{o}C min^{-1}. A nitrogen purge of 50ml/min was used in the oven.

***In vitro* dissolution studies:** *In vitro* drug release [24] from mucoadhesive microspheres was analyzed by using USP dissolution test apparatus 2 (Paddle) with 100 rpm (Disso 2000, Lab India). Predetermined quantities of microspheres were placed in bowel. 900 ml of 0.1 N HCl (pH 1.2) was used as the dissolution media. Dissolution studies were conducted at 37℃±0.2°C. Samples were taken at suitable time intervals and replaced with the same quantity of fresh dissolution medium. Collected samples filtered through 0.45µm syringe, absorbance was measured spectrophotometrically (Shimadzu UV/Visible spectrophotometer 2100; Tokyo, Japan) at 293 nm.

Kinetics of drug release In order to know the drug release mechanism and *in-vitro* drug release kinetics various kinetic models were used. Zero order, first order, Higuchi's, Peppa's models were used in this study and regression coefficient values (R^2) was calculated and analyzed.

Accelerated stability testing According to ICH Q1A (R2): or six months, the optimised formulation (LM 6) was kept in a stability chamber (Remi CHM- 10 S®, India) at 40 2±°C and 75±5% RH and [25] tested for the presence of drugs, mucoadhesiveness, and *in vitro* drug release at 0, 30, and 180 days As checks, samples taken at zero time were used.

***Statistical analysis*:** P< 0.05 was regarded as statistically significant when analysing the data from the production yield, encapsulation efficiency, particle size, *in vitro* release experiments, and *in vivo* studies of microspheres using one-way ANOVA in the GraphPad Prism programme (GraphPad Software).

RESULTS AND DISCUSSION

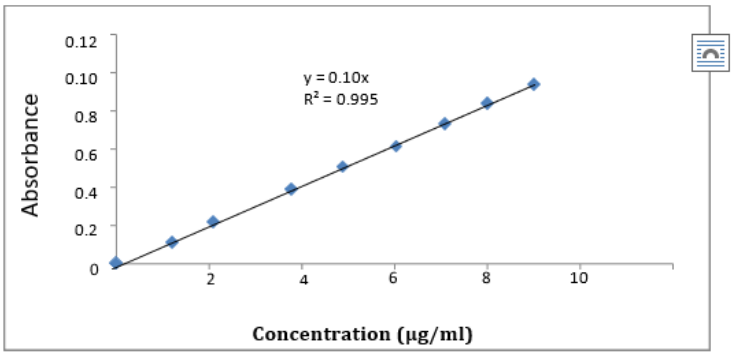


Figure No. 1: Standard curve of Levofloxacin hemihydrate in 0.1 N HCl

Table No. 2: Standard curve of Levofloxacin hemihydrate in 0.1 N HCl

S. No	Concentration (µg/ml)	Absorbance
1	0	0
2	1	0.13
3	2	0.24
4	3	0.34
5	4	0.41
6	5	0.53
7	6	0.62
8	7	0.75
9	8	0.84
10	9	0.995

Values are expressed as mean±S.D (n=3).

Table No. 3: Drug content, Percentage yield and encapsulation efficiency of levofloxacin hemihydrates loaded mucoadhesive microspheres

S. No	Formulation code	Theoretical drug content (%)	Percentage drug loaded	Percentage yield	Practical drug content (%)
1	LCAM 1	55.60	51.77±1.33	32.11±1.25	20.21±1.1
2	LCAM 2	45.45	60.22±0.55	46.51±1.87	22.08±1.33
3	LCAM 3	38.46	68.54±1.03	58.41±1.09	22.27±1.2
4	LCAM 4	55.60	60.25±0.97	55.74±2.15	25.08±1.59
5	LCAM 5	45.45	68.78±1.82	70.61±2.11	26.48±1.35
6	LCAM 6	38.46	73.11±1.49	80.27±1.89	27.21±1.24
7	LCAM 7	55.60	68.55±1.21	61.22±1.87	28.42±1.47
8	LCAM 8	45.45	75.98±1.84	77.64±1.22	29.07±1.63
9	LCAM 9	38.46	78.54±1.55	87.28±1.67	30.77±1.88

Values are expressed as mean±S.D (n=3)

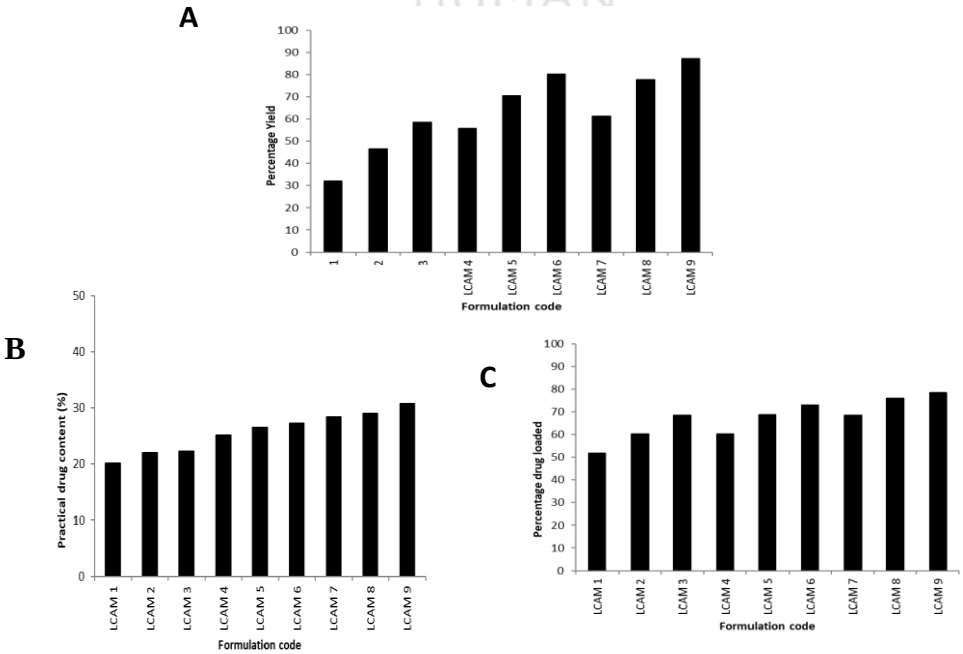


Figure No. 2: a) percentage yield b) Drug content c) encapsulation

Efficiency of levofloxacin hemihydrate loaded Mucoadhesive microspheres.

Table No. 4: Particle Size distribution and mucoadhesiveness evaluation of Levofloxacin Loaded mucoadhesive microspheres

S. No.	Formulation Code	Particle size(µm)	Mucin Adsorption
1	LCAM 1	135.5±3.64	45.81±1.32
2	LCAM 2	217.68±5.87	55.34±1.99
3	LCAM 3	287.24±5.41	65.71±1.32
4	LCAM 4	229.22±4.55	55.03±1.87
5	LCAM 5	314.28±4.23	68.58±1.23
6	LCAM 6	397.55±3.28	79.41±0.86
7	LCAM 7	284.37±4.98	62.90±1.41
8	LCAM 8	357.23±5.01	73.11±1.59
9	LCAM 9	448.59±5.44	82.97±0.82

Values are expressed as mean±S.D (n=3).

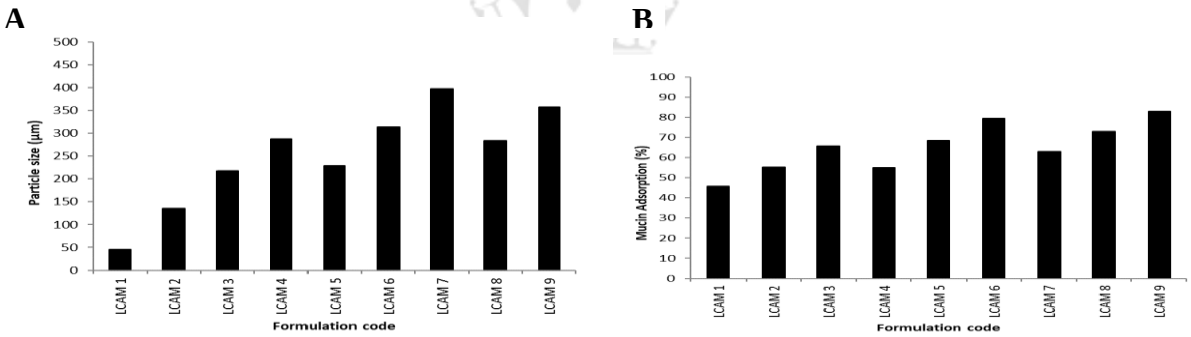


Figure No. 3: A) Particle Size distribution B) Mucoadhesiveness evaluation of Levofloxacin Loaded mucoadhesive microspheres

SEM stands for scanning electron microscopy. The shape and surface characteristics of the microspheres were examined using a SEM, or scanning electronic microscope. HITACHI-SEM MODELS – 450 model scanning electron microscope was used for the study. The prepared microspheres were placed directly on to the SEM sample holder by using double-sided fixing tape and coated with gold film (thickness 200 nm) under reduced pressure (0.001 tort) and photographed. The SEM microspheres show a spherical structure with a rough surface morphology. Some of microsphere showed good carrier property and good

floating ability. The SEM analysis shows the development of levofloxacin loaded alginate microsphere of suitable properties for sustained and control release of the drug.

The results revealed that the increasing in the concentration of sodium alginate increase the size of the beads based on the fact that sodium alginate binds more calcium chloride by cross linking. These observations are in accordance with research study which described that higher viscosity resulted from increase in the alginate concentration causes greater drug entrapment due to high degree of cross linking.

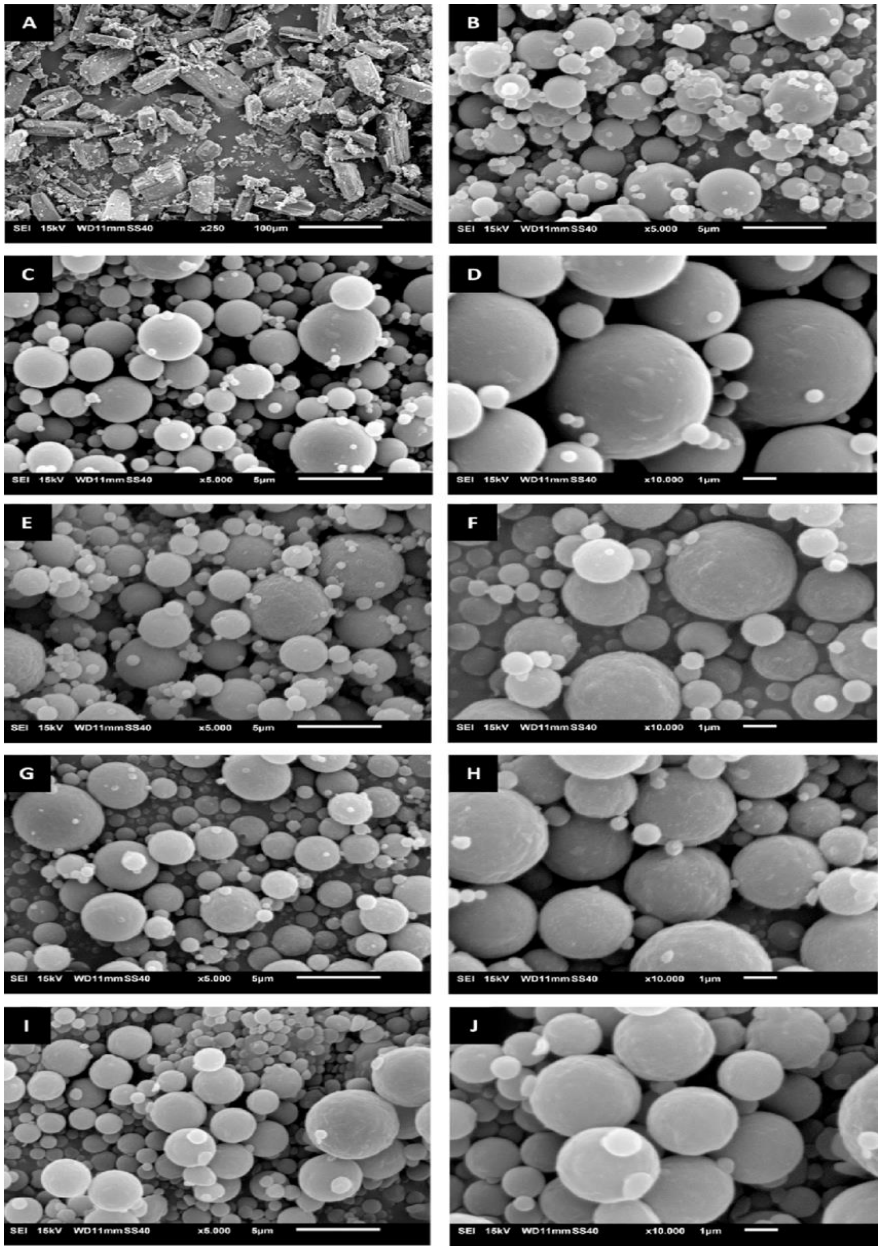


Figure No. 4: Scanning Electron Micrograph of Levofloxacin Hemihydrate Loaded Mucoadhesive Microspheres

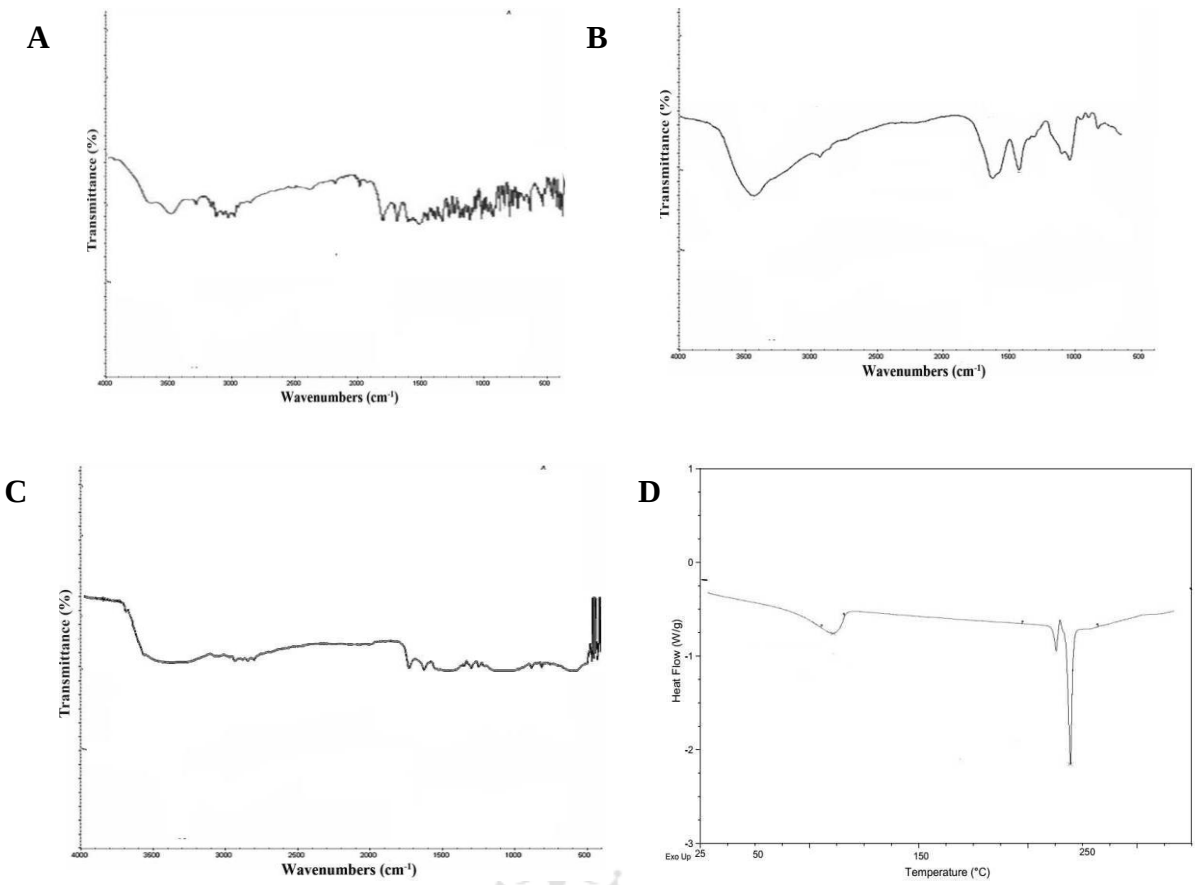


Figure No. 5: Compatibility studies by FTIR A) FTIR spectra of Levofloxacin hemihydrate B) FTIR spectra of Blank mucoadhesive microspheres C) Characteristic IR bands of Levofloxacin hemihydrate in mucoadhesive microspheres. D) DSC spectra of Levofloxacin hemihydrate

The DSC thermogram of LVF showed endothermic transitions at 94.2℃ and 237.2 ℃ due to the decomposition of LVF.

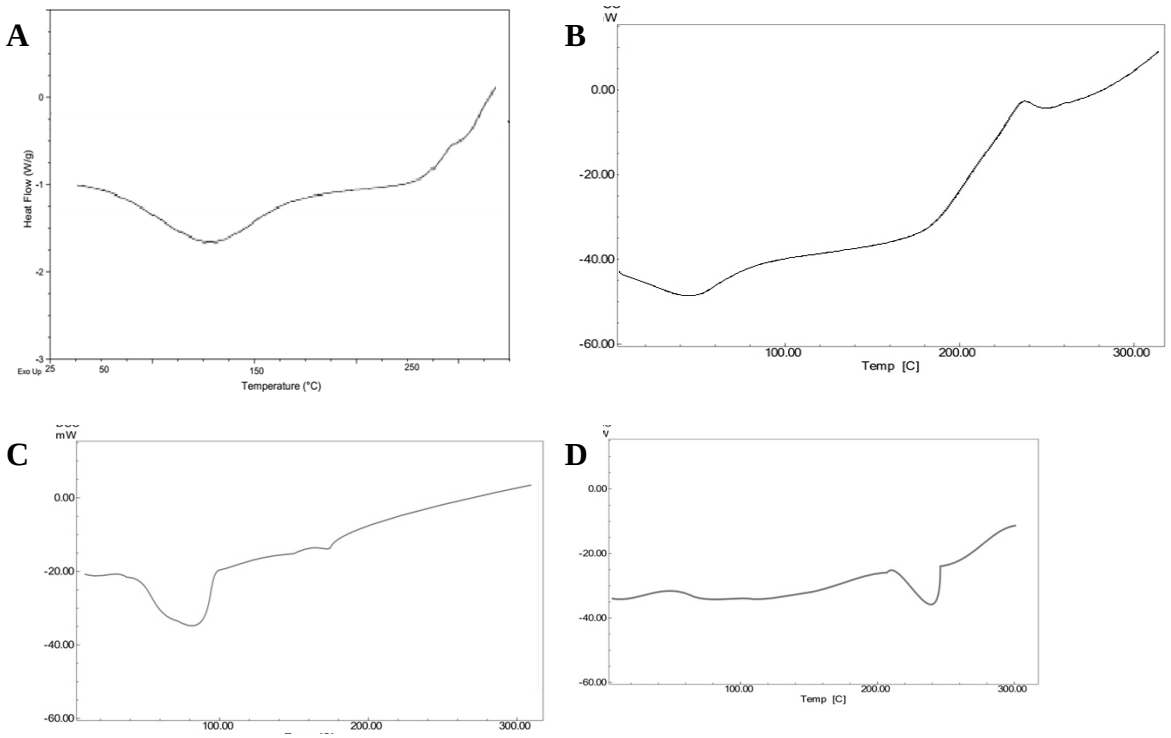


Figure No. 6: A) DSC spectra of Dsc of Sodium alginate B) DSC spectra of DSC of Chitosan C) DSC spectra of blank microspheres D) DSC spectra of Levofloxacin hemihydrate loaded mucoadhesive microspheres [LCAM5]

***In Vitro* Dissolution Studies**

Table No. 5: *In vitro* release profile of Levofloxacin hemihydrate loaded mucoadhesive microspheres (Formulation LCAM1)

Time (hours)	LCAM1	LCAM2	LCAM3	LCAM4	LCAM5	LCAM6	LCAM7	LCAM8	LCAM9
1	0	0	0	0	0	0	0	0	0
2	54.67±1.87	46.22 ± 1.45	41.22±1.55	47.42	38.22±1.98	33.98±1.47	33.41±1.78	28.33±1.94	25.87±1.55
3	81.65±1.14	71.87 ±1.87	64.82±1.91	66.04±1.11	56.21±1.02	50.88±1.98	53.55±2.09	43.98±1.99	41.22±2.98
4	99.51±081	93.24 ±1.55	85.01±1.87	4.21±1.68	65.99±2.57	61.55±1.47	68.87±1.10	55.88±2.57	49.23±2.11
5	-	99.88 ±0.54	95.22±1.91	99.42±0.84	76.24±1.58	69.04±2.07	84.28±1.82	65.24±5.44	57.19±1.34
6	-		99.66±0.58		87.68±2.23	79.24±1.98	99.68±0.87	75.87±1.98	65.55±1.98
7	-				99.65±0.87	86.21±1.58		87.21±1.17	73.87±1.58
8	-					95.98±0.78		99.74±0.55	87.22±1.85
9									

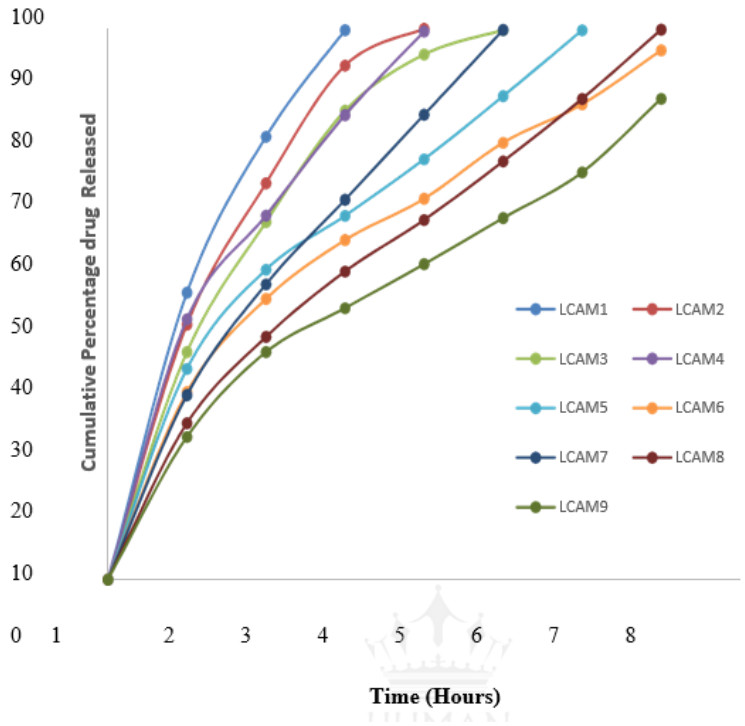
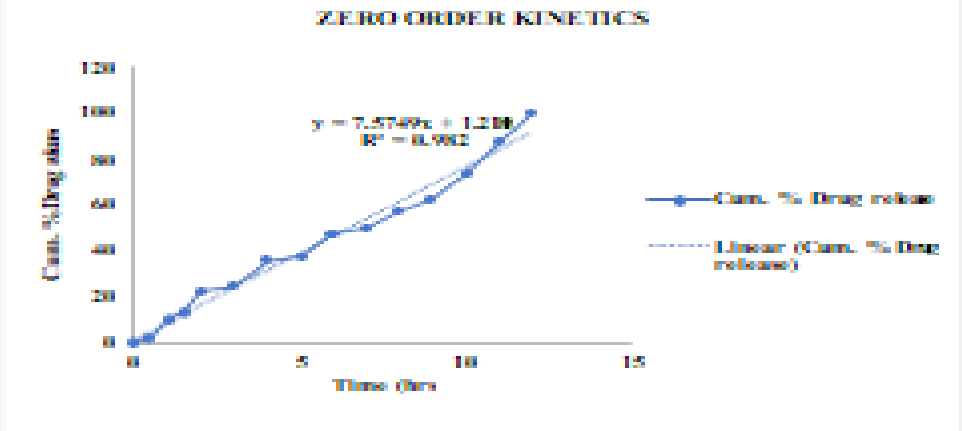


Figure No. 7: Drug release pattern of various formulations of levofloxacin Hemihydrate

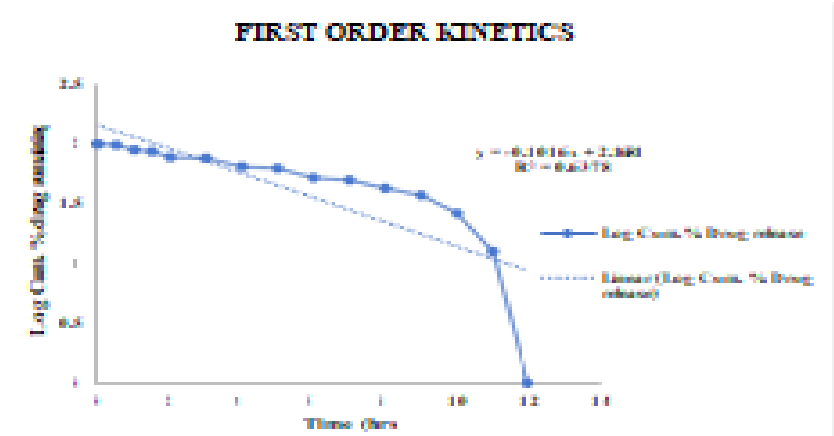
Table No. 6: *In vitro* release kinetic data of Levofloxacin hemihydrate loaded mucoadhesive microspheres

F Code	Zero order plot		First order plot		Higuchi plot	Korsemeyer Peppa's plot	
	K_0	R^2	K_1	R^2	R^2	n	R^2
LCAM 1	19.4289	0.9957	-0.4582	0.8178	0.9913	-	-
LCAM 2	18.243	0.9911	-0.59788	0.7878	0.9971	-	-
LCAM 3	13.211	0.9981	-0.2042	0.9253	0.9911	0.6186	0.9991
LCAM 4	19.574	0.9925	-0.5478	0.7784	0.9987	-	-
LCAM 5	15.447	0.9971	-0.6845	0.7257	0.9908	0.5381	0.9964
LCAM 6	14.369	0.9960	-0.2978	0.7875	0.9982	0.6421	0.9909
LCAM 7	20.281	0.9985	-0.6187	0.7921	0.9965	-	-
LCAM 8	12.348	0.9801	-0.1841	0.9854	0.9944	0.5841	0.9955
LCAM 9	10.212	0.9946	-0.0975	0.9125	0.9908	0.5245	0.9981

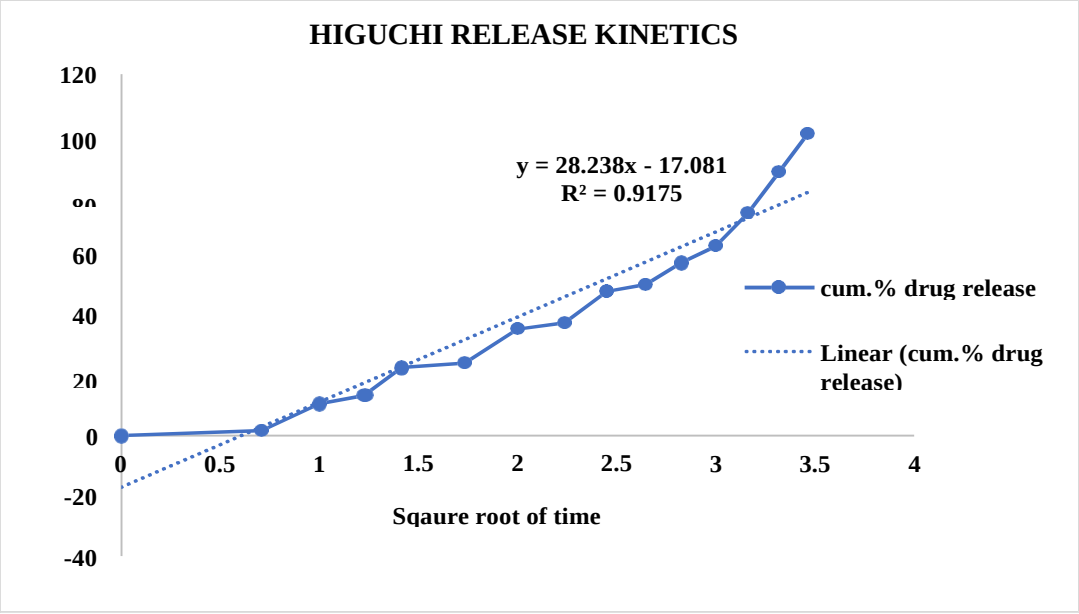
K_0 – Zero order rate constant **K_1** – First order rate constant **R^2 –** Regression coefficient **n** Diffusion exponent



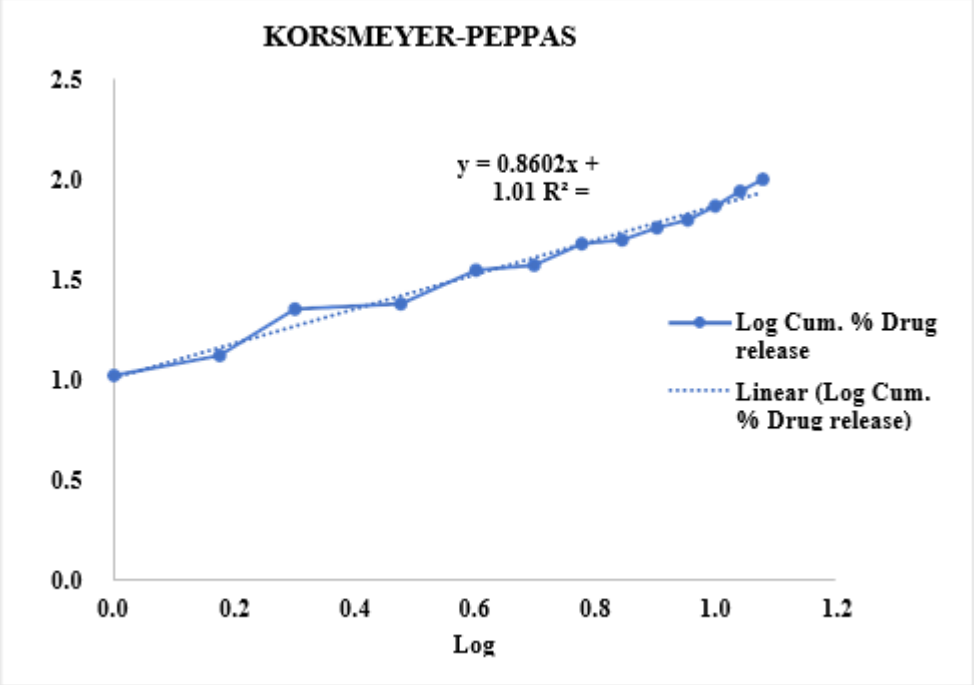
A) A plot of Zero order kinetics of LCAM5



B) A plot of First order kinetics of LCAM5



C) A plot of Higuchi release kinetics of LCAM5



D) A plot of Korsmeyer-Peppas kinetics of LCAM5

Figure No. 8: Drug release kinetics of optimized formulation LCAM5

Accelerated Stability Studies

Table No. 7: Accelerated stability data of Levofloxacin hemihydrate loaded mucoadhesive microspheres (Formulation LCAM5) [Tested according to ICH Q1A (R2)]

S. No.	Time (days)	Mucoadhesive strength	Drug content (%)	Drug release (%)
1	Before storage (0 day)	68.74±1.45	26.27±1.43	99.62±1.87
2	30 days (After storage*)	68.01±1.92	25.94±1.84	99.82±1.84
3	90 days (After storage*)	67.98±1.74	25.68±1.52	99.64±1.13
4	180 days (After storage*)	67.14±1.84	25.80±1.01	98.03±1.51
P -Value		0.0345	0.0387	0.0411

*Storage at 40°C and 75% RH [n = 3]

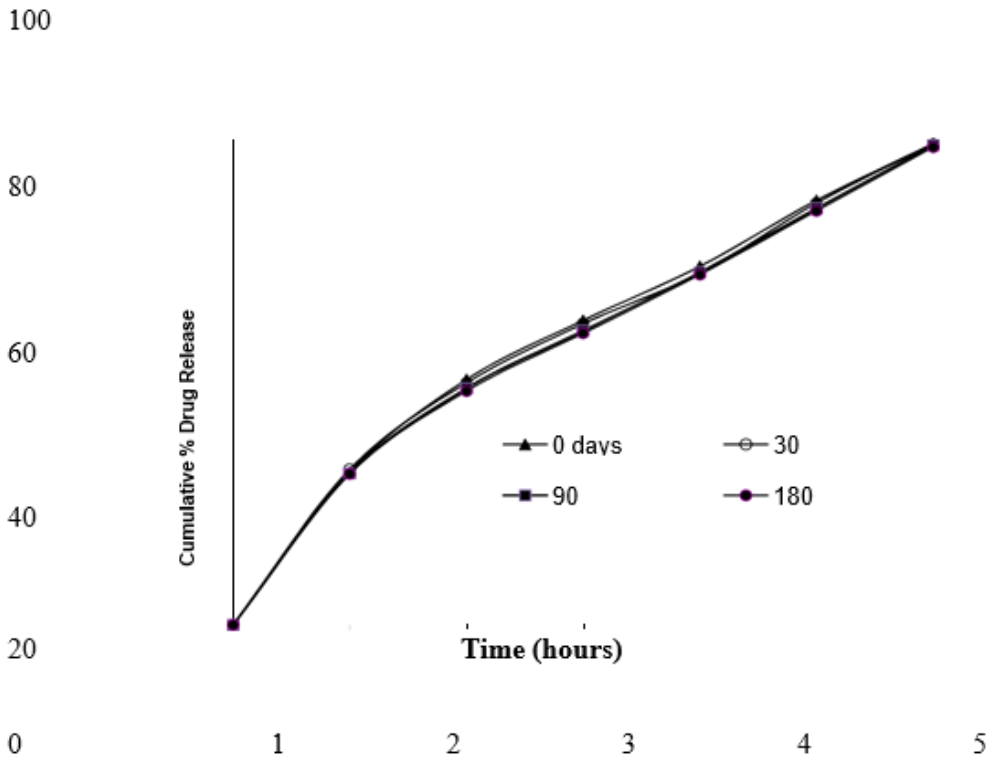


Figure No. 9: Accelerated stability data of Levofloxacin hemihydrate loaded mucoadhesive microspheres (Formulation LCAM5

DISCUSSION:

In this study levofloxacin hemihydrate mucoadhesive microspheres were prepared by ion gelation method. Various concentration of sodium alginate and calcium chloride was used along with chitosan to investigate the effect of parameter on percentage yield, particle size, mucoadhesiveness, surface morphology of microspheres and drug release. Levofloxacin hemihydrate standard curve was plotted at 370°C in 0.1 N HCl with a pH of 1.2.

Percentage yield of microspheres: Levofloxacin hemihydrate loaded microspheres had a percentage yield that ranged from 32.11±1.25% to 87.28±1.67%.

The output of microspheres rose as calcium chloride and sodium alginate concentrations were increased. It is obvious that lowering the polymer concentration has caused the percentage yield to drop.

Drug content and encapsulation efficiency: Levofloxacin hemihydrate-loaded microspheres ranged in drug concentration from 20.21±1.01% to 30.77±1.88%. The prepared

microspheres' encapsulation effectiveness ranged from 51.77±1.33% to 78.54±1.55%. By gradually increasing the concentration of calcium chloride and sodium alginate, the encapsulation efficiency increased.

As the alginate concentration rose, a higher loading efficiency was attained.

Particle size analysis- Shape and surface characterization

One of the most crucial factors in the creation of microspheres has to do with the viscosity of the polymer solution.

Levofloxacin hemihydrate laden microspheres were found to have a mean diameter that ranged from 135.5±3.64 µm to 448.59±5.44µm.

Based on the notion that sodium alginate binds more calcium chloride by cross-linking, the results showed that increasing the concentration of sodium alginate increased the size of the beads.

***In vitro* Evaluation of Mucoadhesiveness**

The *in vitro* mucoadhesiveness investigation was carried out on all the produced microcapsule formulations. The produced microspheres' mucoadhesive properties ranged from 45.81±1.32% to 82.97±0.82%. It was discovered that increased polymer concentrations result in a higher mucoadhesive property. The contact stage and the consolidation stage are the two stages that make up the mucoadhesion mechanism. The mucoadhesive's initial contact with the mucous membrane, along with the formulation's subsequent swelling and spreading, marks the beginning of its deep engagement with the mucous layer. The presence of moisture during the consolidation stage activates the mucoadhesive materials. Chitin is alkaline deacetylated to produce chitosan, a linear polymer of D-glucosamine. Numerous investigations have demonstrated that the charged amino group of the D-glucosamine residues in chitosan may interact with the gastric mucus's N-acetylnuraminic acid (sialic acid) by electrostatic contact, prolonging the substance's duration in the stomach. The combination of these two polymers gives microspheres enhanced mucoadhesive properties.

Compatibility studies: To find interactions between the medication and the excipients, DSC and FT-IR tests were conducted on the raw ingredients and the microspheres.

FTIR studies For pure drug, drug-loaded microspheres, and blank microspheres, FTIR spectra were taken. All of the aforementioned peaks of levofloxacin hemihydrate were also visible in the drug-loaded formulations' FTIR spectra, albeit with a minor broadening and intensity reduction. This confirms that the drug is present in the polymer without interacting with other molecules.

Differential Scanning Calorimeter [DSC] studies: For pure drug, drug-loaded microspheres, and blank microspheres, DSC spectra were taken. At roughly 238.5°C, levofloxacin hemihydrate reaches its endothermic peak. The mixing procedure, which reduces the purity of each component in the combination, can be blamed for the modest change in peak shape and minimal broadening seen in the DSC thermogram of the optimized formulation-[LCAM5]. Levofloxacin hemihydrate loaded microspheres showed no endothermic peak matching to the hemihydrate-of the drug. The medicine was molecularly disseminated in the microspheres, as evidenced by the absence of crystalline domains in the microcapsules, according to Department of Pharmaceutics.

***In vitro* dissolution studies:** A common quality control technique to assess drug release from oral dosage forms is the dissolution test. The tight connection between the glucuronic acid residues is the basis for sodium alginate and calcium chloride's cross-linking or gelation process. Due to increased crosslinking that created a more stiff gel network and thus better sustained release properties, beads made with 3% w/v calcium chloride exhibited the greatest sustained release effect. Additionally, it was clear from the literature that when the calcium chloride concentration of the solution grew, the diffusion of the drug from the alginate matrix reduced, most likely as a result of increased cross-linking with sodium alginate.

***In vitro* drug release and kinetics of release:** The formulations displayed reasonably good linearity when the release data of levofloxacin hemihydrate loaded microspheres were plotted according to the first order equation, with an R2 value of 0.7878-0.9854, whereas the same data improved the R2 value of 0.9801-0. 0.9985 When the data were plotted according to the zero order equation. Higuchi's equation could best be used to describe the *in vitro* release patterns of levofloxacin hemihydrate from all of the formulations in our experiment since the plots exhibited high linearity with an R2 value of 0.9908– 0.9982.

CONCLUSION

The melting point of Levofloxacin Hemihydrate was found to be 214 - 216 ℃. The regression coefficient (R^2) value of found for Levofloxacin Hemihydrate calibration curve developed in 0.1 N HCl [pH 1.2] at 37℃. The standard calibration curves are linear over the concentration ranges from Levofloxacin hemihydrate: 0.1 g/ml to 10 g/ml [R2 =0.9957]. By utilising polymers and the ionotropic gelation process, microspheres were created.

Sodium alginate, chitosan, calcium chloride, hydroxy propyl methyl cellulose etc. formulated microspheres were subjected for evaluation parameters such as particle size, Drug content from 20.21±1.01% to 30.77±1.88%, encapsulation efficiency from 51.77 ± 1.33% to 78.54± 1.55%, mucoadhesive property from 45.81±1.32% to 82.97±0.82%. From *in vitro* Drug release of various formulations was studied, the drug release from formulation F5 Was found to be at maximum percentage i.e., 99.42% and showed controlled release for 6-8 hrs. Hence it may achieve the aim of controlling the drug release as the plots of zero order model was linear. In conclusion prolong retention time in GIT and reduced frequency of dosing, enhanced bioavailability of levofloxacin resulting from the prepared floating microspheres, could contribute to the provision of anti-Bacterial agents. Formulation LCAM 5 was found to be Best formulation based on Particle size, Dissolution rate and mucoadhesive property. It shows Prolong retention time in GIT and reduces the frequency of dosing. The mucus turnover rather than the mucus- polymer interaction that controls the presence of mucoadhesive formulations through the GIT. [Formulation LCAM 5 consisting of 2% w/v Sodium alginate, 1% w/v chitosan and 3% w/v Calcium chloride]. Accelerated stability studies were conducted for formulation LCAM 5.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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