DESIGN AND DEVELOPMENT OF LEVOFLOXACIN HEMIHYDRATE GASTRORETENTIVE DRUG DELIVERYSYSTEM OF FOR H. PYLORI INFECTION

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Abstract

Aim:To prepare mucoadhesive microspheres for the treatment of H.Pylori infection.

Methods: Mucoadhesive microspheres were prepared by emulsion solvent evaporation method. To achieve the gastro retentiveactivity in this study by blending polymers.

Results: To achieve the gastroretentive property in this study, a combination of polymers, such asChitosan and HPMC K15M were used. The percentage yield for LVX loaded microspheres were found to be in the range of 55.84 % to 61.56 %,respectively. The drug content of the LVXloaded microspheres varied from 49.23% to 61.23%. The encapsulation efficiency of the prepared microspheres varied from 58.52% to 65.76%.The particle size of microspheres of all the formulations ranged from220.31 µm to 302.46 µm. Average Mucoadhesionin the percentage of all the formulations was found to range from 67.85 % to 91.03 %. Drug release was retarded by increasing the proportion of chitosan and HPMC K15 M, respectively. The slope of the regression line from the Higuchi plot indicates the rate of drug release and thus confirms that the mode of release was diffusion.No remarkable changes were observed in drug content, mucoadhesiveness and in vitro drug release in stability studies.

Introduction

Helicobacter pylori (H. pylori), or formally known as Campylobacter pylori, is a Gram-negative, micro-aerophilic, spiral microorganism that can colonize the healthy stomach lining is associated with gastritis, peptic ulcer disease, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer.[1–3] The direct transmission from person to person, either oral or fecal-oral route or both, is expected to lead to new infection. In patients with duodenal ulcers, the inflammation of the gastric mucosa induced by the infection is most pronounced in the non–acid-secreting antral region of the stomach and stimulates the increased release of gastrin.[4]As with duodenal ulcers, eradicating the infection usually cures the disease, provided that the gastric ulcer is not due to NSAIDs. Gastric cancers (i.e., those distal to the gastroesophageal junction).[5]The risk of cancer is highest among patients in whom the infection induces inflammation of both the antral and fundic mucosa.

H. pylori infection and the presence of gastric MALT lymphomas eradication of the infection causes regression of most localized gastric MALT lymphomas.[6]The prevalence of H. pylori infection is lower among patients with gastroesophageal reflux disease (GERD) and those with oesophagal adenocarcinoma. A recent meta-analysis showed no significant association between H. pylori eradication and an increased risk of GERD.[7] Bismuth-based quadruple therapy (BQT), concomitant/non-bismuth quadruple therapy, and clarithromycin-based triple therapy were recommended first-line options in the 2017 ACG guideline. Bismuth has a synergistic effect with several antibiotics that is independent of clarithromycin and metronidazole resistance. In patients who fail first-line treatment, BQT or levofloxacin-based triple therapy are second-line options that avoid the re-use of clarithromycin. Levofloxacin-based triple therapy should only be used the second line if Levofloxacin was not used first-line.[8]

Compliance, antibiotic resistance, disease entity associated with the H. pylori infection, bacterial virulence factors and pharmacological properties. Compliance is a crucial element for successful H pylori eradication. Antibiotic resistance is the

primary cause of treatment failure, besides poor compliance. Antibiotic resistance concerns mainly two of the major antibiotic components in current eradication regimens: macrolides and nitroimidazoles.[9]Within the group of macrolides there is almost 100% cross-resistance between different compounds, including clarithromycin, azithromycin and roxithromycin.The same applies to the two principal nitroimidazoles: tinidazole and metronidazole. Disease entities associated with H pylori infection have been recognized to influence treatment efficacy. Peptic ulcer patients tend to respond better to eradication therapy than patients with functional dyspepsia.[9-10]

MATERIALS AND METHODS

Materials

Levofloxacin hemihydrate was purchased from GoldsunPharmaceuticalslimited, Mumbai, Chitosan and HPMCK15Mwas purchased from Macleods Pharmaceuticalslimited Mumbai,Mumbai. Lightliquidparaffin, Heavy liquid paraffin, Petroleum ether and Span80 was purchased from SDFinechemicals,Mumbai, Methanol was purchased from Merckspecialtiespvt.Ltd.Mumbai.

Formulationofmucoadhesivemicrospheres[11]

A fixed weight (1 g), but at varied proportions, of Chitosan and HPMC K15 was dissolved in a fixed volume (30 ml) of (but at varied proportions)1% acetic acid solution at room temperature. Weighed amount of Levofloxacin hemihydrate and chitosan was added to the polymer's solution. The prepared organic phase was introduced dropwise into 300 ml of liquid paraffin (150ml Heavyliquid paraffin+150ml Lightliquid paraffin) containing Span 80 (0.2% v/w) undera mechanical stirrer for 2 hrs at 80°C to allow the solvent to evaporate and the microspheres were collected by filtration. The microspheres were washed repeatedly with petroleum ether until freed from oil and dried for 24 hr at room temperature.[12-14]

Table No: 1 Formulation of mucoadhesive microsphere

Formulation Code	Polymer ratio (Chitosan: HPMC K15M)	Stirring Speed	Levofloxacin hemihydrate (% w/v)
F1	1:1	1000 rpm	5
F2	1:2	1000 rpm	5
F 3	1:3	1000 rpm	5
F4	1:4	1000 rpm	5
F5	2:1	1000 rpm	5
F6	2:2	1000 rpm	5
F7	2:3	1000 rpm	5
F8	2:4	1000 rpm	5
F9	3:1	1000 rpm	5
F10	3:2	1000 rpm	5
F11	3:3	1000 rpm	5
F12	3:4	1000 rpm	5

Evaluation of mucoadhesive microspheres

Compatibility studies

Fourier-Transform Infrared Spectrophotometer (FTIR)[15]

Infrared spectra for pure Levofloxacin hemihydrate, blank microspheres, Levofloxacin hemihydrate mucoadhesive microspheres were obtained on a FTIR-[Shimadzu (84005)] spectrophotometer using the potassium bromated diskmethod.200mg potassium bromate wasusedfortheanalysisof2mg of the sample. The scanning range was setinto450–4000cm-1.

Determination of percentage yield of microspheres [16]

Dried microspheres were collected and weighed accurately using a digital balance. The percentage yield of prepared microspheres was calculated by using the formula mentioned below:

$$\text{PercentageYield} = \frac{\text{Mass of microspheres obtained}}{\text{Total weight of drug and polymer used}} \times 100$$

Determination of drug content and encapsulation efficiency[17-18]

The drug content of the microspheres was measured by extraction method. Accurately weighed 5mg of mucoadhesive microspheres were crushed into a powder using glass mortar and pestle. The resulting powdered mucoadhesive microspheres were dispersed in 15 ml of 0.1N HCL solution. The final suspension was vortexed at 2500 rpm for 1 minute and then for afurther2hoursat1000rpmandat room temperature. In order to calculate the amount of drug entrapped in the mucoadhesive microspheres, the suspension was then filtered through a 0.45µm syringe filter and the filtrate was analyzed by UV. [17]

$$\% \text{ Drug content} = \frac{\text{mass of drug in microspheres}}{\text{Mass of microspheres}} \times 100$$

For the determination of encapsulation efficiency following formula is used

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

Particle size analysis[19]

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

Shape and surface characterization [20]

The shape and surface characteristics of the microspheres were observed under a Scanning Electron Microscope (SEM). HITACHI-SEMMODEL 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(thickness200nm)under reduced pressure(0.001torr)and photographed.

In vitro evaluation of mucoadhesiveness[21-23]

The in-vitro wash off test was carried out to evaluate the mucoadhesive potential of the microspheres. A small portion of the sheep intestinal mucosa was mounted on a glass slide and accurately weighed microspheres were sprinkled on the mucosa. Around 100microspheres were spread on the wet mucosa and the prepared slide was hung onto one of the grooves of the USP tablet disintegrating test apparatus filled with 0.1N HCL giving regular up and down movements for 60 minutes. At the end of 60 min, numbers of microspheres still adhering to the intestinal mucosa were counted. The percentage of mucoadhesion was calculated by following formula

$$\% \text{ Mucoadhesion} = (Wa\text{-}Wl) \text{ X } 100 / Wa$$

Where,

Wa= weight of microspheres applied

Wl= weight of microspheres leached out

In vitro dissolution studies[24-26]

In vitro drug release from mucoadhesive microspheres was analyzed by using USP dissolution test apparatus 2 (Paddle) with a stirrer at 100 rpm (Disso 2000, Labindia). Predetermined quantities ofmicrospheres were placed in the bowl. 900 ml of 0.1N HCl (pH 1.2) wasused as the dissolution media. Dissolution studies were conducted at 37°C. Samples were taken at suitable time intervals and replaced with the same quantity off reshdissolution medium. Collected samples filtered through 0.45µm syringe absorbance was measured spectrophotometrically (Shimadzu UV Visiblespectrophotometer2100;Tokyo,Japan).

Kinetics of drug release [27-29]

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 [23-24] models were used in this study and regression coefficient values (R2) was calculated and analyzed.

Accelerated stability testing according to ICHQ1A(R2) [30-32]

The optimized formulation was stored inastability chamber (Remi CHM- 10 S®, India) at 40 ± 2°C and humidity of 75 ±5%RH for 6 months and examined for the drug content, mucoadhesiveness and in vitro drug release0, 30, 90, and 180 days. The zero time samples were used as controls.

RESULT AND DISCUSSION

Compatibility studies

Fourier Transform Infrared Spectrophotometry (FTIR) Studies

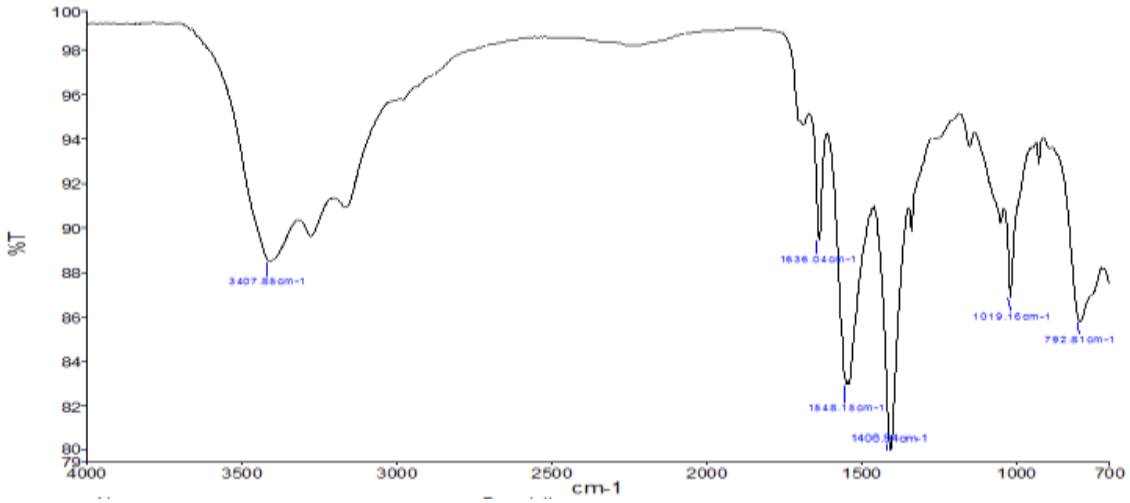

Figure 1: FTIR spectra of Blank Mucoadhesive microsphere

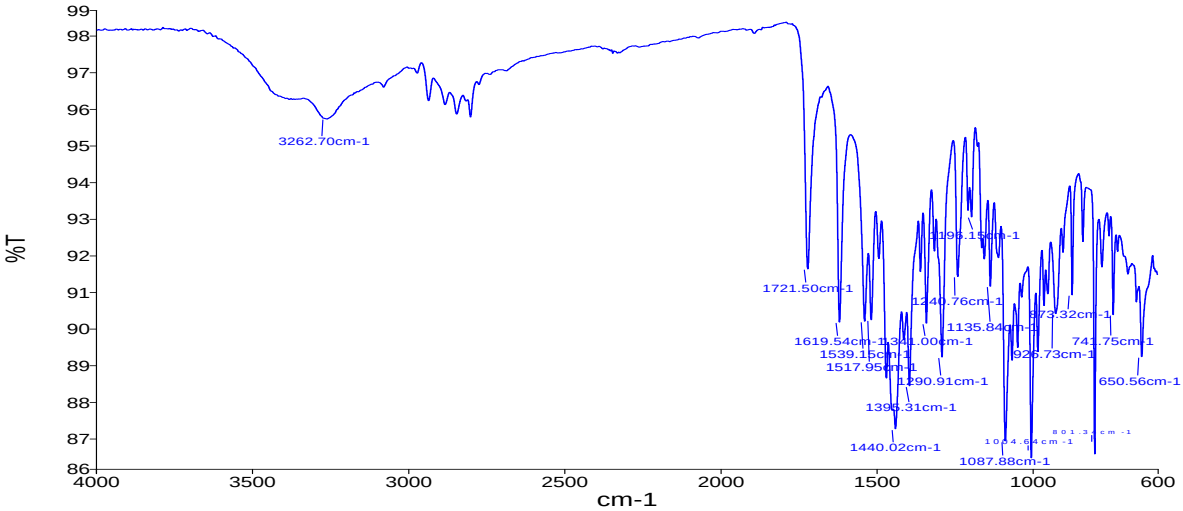

Figure 2: FTIR spectra of drug loaded Mucoadhesive microspheres

FTIR spectra were recorded for drug-loaded microspheres and blank microspheres. According to the FTIR analysis results, excipient and active substances did not interact. All the peaks given in below table of levofloxacin hemihydrate were also present in FTIR spectrum of drug-loaded microspheres with slight broadening and reduction in intensity in drug-loaded formulations that confirm the presence of drug in the polymer without any interaction.

Table 2: FTIR study for blank and final formulation

S. No	Types of bond	Actual frequency (Cm^{-1})	Observed Frequency	
			Blank formulation (Cm^{-1})	Final formulation (Cm^{-1})
1	–COOH (Acid)	3700-3100	3462.57	3410.32
2	–CH3 (Alkane)	2850-2960	2369.10	2908.11
3	C=O (Ketone)	1730-1760	1738.26	1746.78
4	N-H (amide)	3000-3500	3257.42	3467.86

Percentage yield

Table 2: Percentage yield of Levofloxacin hemihydrate loaded mucoadhesive microspheres

S.No	Formulation code	Percentage yield
1	F1	56.16
2	F2	59.39

3	F 3	58.15
4	F4	59.94
5	F5	60.49
6	F6	61.06
7	F7	60.18
8	F8	61.38
9	F9	59.24
10	F10	61.56
11	F11	60.83
12	F12	55.84

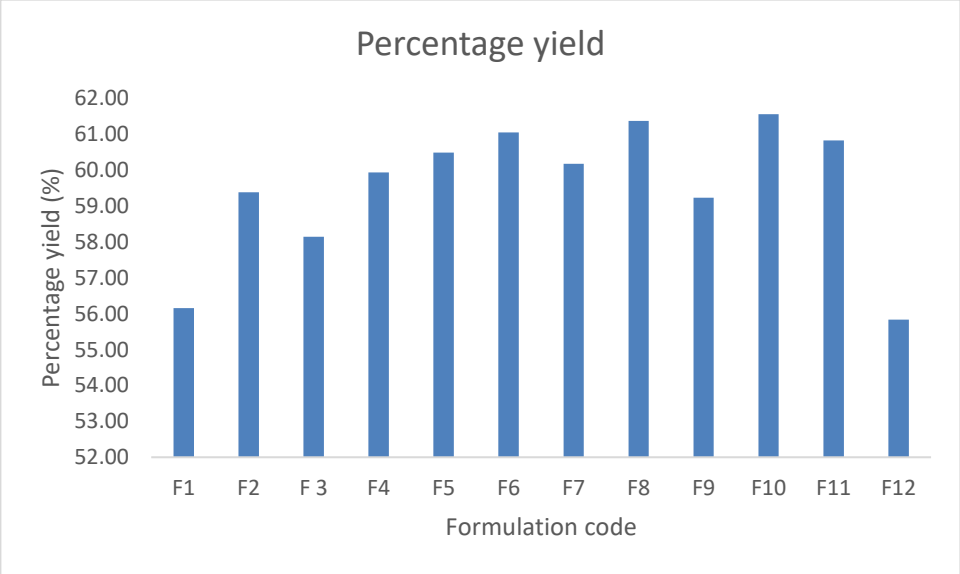

Figure 3: Percentage yield of Levofloxacin hemihydrate loaded mucoadhesive microspheres

Percentage yield of Levofloxacin hemihydrate loaded mucoadhesive microspheres different formulations F1-F12, were calculated and the values rangesfrom 55.84 % to 61.56 %. The results were tabulated in the above table. This higher percentage yields indicates that this Emulsion Solvent Evaporation method was very useful for adoption in the formulation of Levofloxacin mucoadhesive microspheres.

Encapsulation efficiency

Table 3: Encapsulation efficiency of levofloxacin hemihydrate loaded mucoadhesive microspheres

S.No	Formulation code	Encapsulation Efficiency(%)
1	F1	63.49
2	F2	61.42
3	F 3	61.51
4	F4	64.21
5	F5	65.76
6	F6	65.19
7	F7	62.46
8	F8	63.73
9	F9	61.43

10	F10	59.75
11	F11	58.52
12	F12	60.69

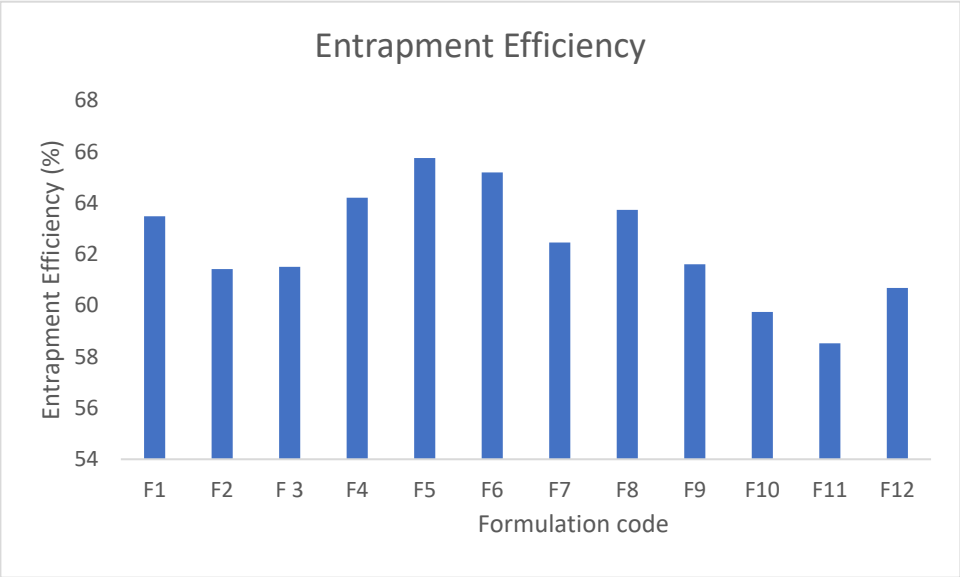

Figure 4: Encapsulation efficiency of levofloxacin hemihydrate loaded mucoadhesive microspheres

Encapsulation efficiency of levofloxacin hemihydrate loaded mucoadhesive microspheres58.52% to 65.76%. The encapsulation efficiency increased progressively by increasing the concentration of polymer. Higher efficiency was obtained as the concentration of a polymer increased. The stirring rate also caused a significant decrease of entrapment efficiency. Increased polymer proportion leads to hardening of droplets, results in reduced drug diffusion into the medium.

Drug Content

Table 4: Drug content of levofloxacin hemihydrate loaded mucoadhesive microspheres

S. No	Formulation code	Drug content (%)
1	F1	59.48
2	F2	58.72
3	F 3	55.63
4	F4	60.17
5	F5	61.23
6	F6	55.62
7	F7	58.69
8	F8	56.15
9	F9	54.82
10	F10	49.23
11	F11	52.64
12	F12	56.17

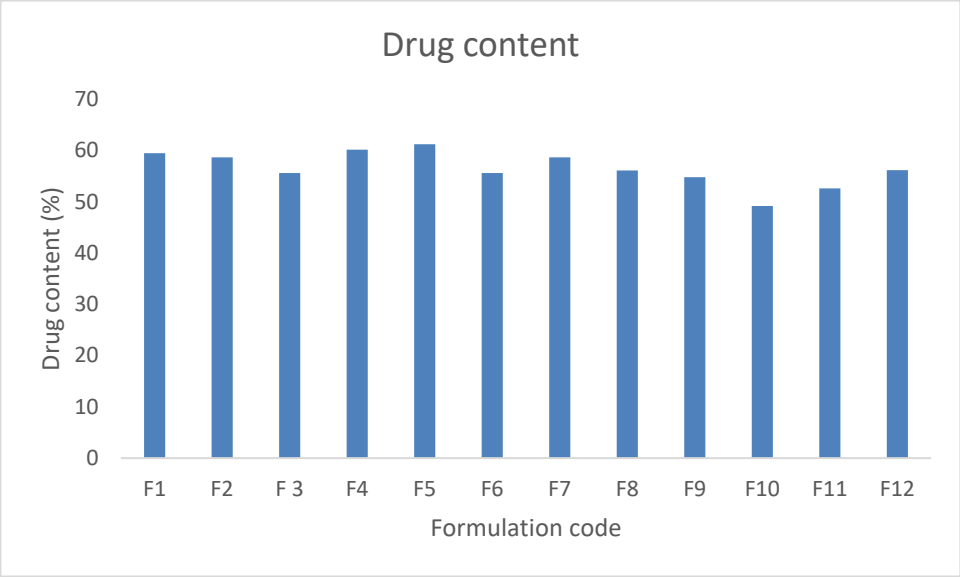

Figure5: Drug content of Levofloxacin hemihydrate loaded mucoadhesive microsphere

Drug content of levofloxacin hemihydrate loaded mucoadhesive microspheres ranges from 49.23% to 61.23%. Higher loading efficiency was obtained as the concentration of polymer increased. It was observed that drug content was found to be directly proportional to polymer concentration. This may be attributed to the greater availability of active binding sites in the polymeric chains and consequently, the greater degree of crosslinking as the quantity of polymer is increased.

PARTICLE SIZE ANALYSIS

Table 5: Particle size analysis of Levofloxacin hemihydrate loaded mucoadhesive microspheres

S.No	Formulation code	Particle size(µm)
1	F1	273.32
2	F2	279.16
3	F 3	274.48
4	F4	298.53
5	F5	302.46
6	F6	299.17
7	F7	264.61
8	F8	269.15
9	F9	284.26
10	F10	274.38
11	F11	220.31
12	F12	256.25

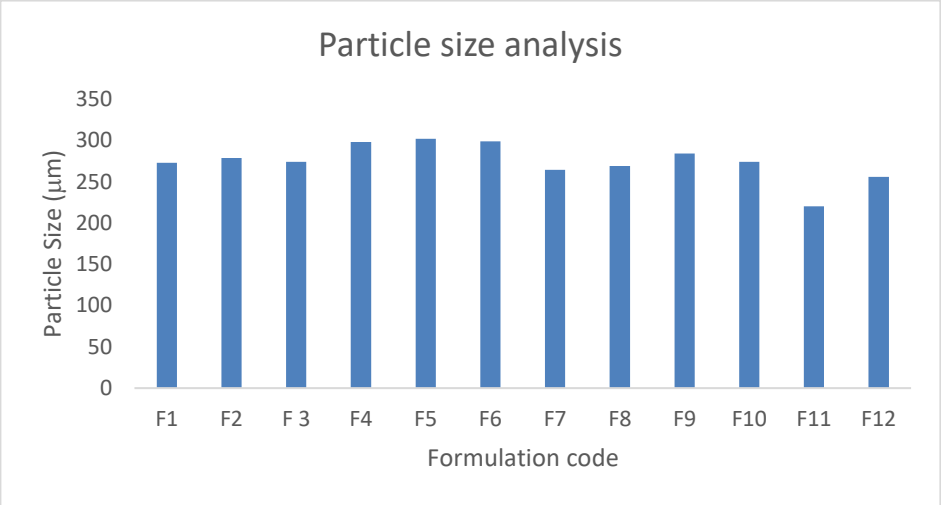

Figure 6: Particle size analysis of Levofloxacin hemihydrate loaded mucoadhesive microsphere

Particle size analysis of Levofloxacin hemihydrate loaded mucoadhesive microsphere 220.31 µm to 302.46 µm.With increase in chitosan concentration in the microspheres, the particle size also improves, which could be due to an increase in the viscosity of drug and polymer proportion and thickness of polymer. These observations could be attributed to the fact that an increase in organic phase viscosity would hold the drug firmly inside the microsphere so that the diffusion of the drug is slowed. Also, an increase in the viscosity could decrease the porosity of the microspheres.

Shape and surface characterization

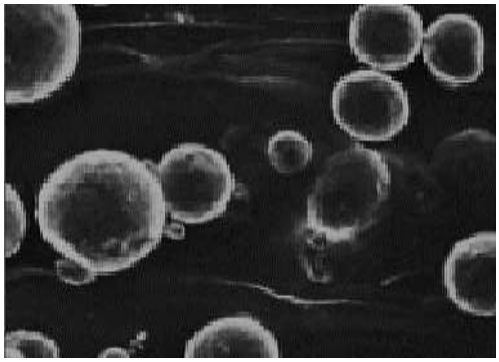

Figure 7: SEM photograph of levofloxacin hemihydrate formulation

Image of Microspheres is presented in the above figure, which revealed that microspheres were almost spherical in nature with slight smooth surface morphology.

In vitro evaluation of mucoadhesiveness

Table 6: Mucoadhesion of Levofloxacin hemihydrate loaded microspheres

S.No	**Formulation code**	**MMucoadhesion (%)**
1	F1	75.97
2	F2	78.65
3	F 3	80.47
4	F4	85.01
5	F5	88.25
6	F6	91.03
7	F7	82.54
8	F8	76.33

9	F9	67.85
10	F10	73.65
11	F11	78.34
12	F12	76.2

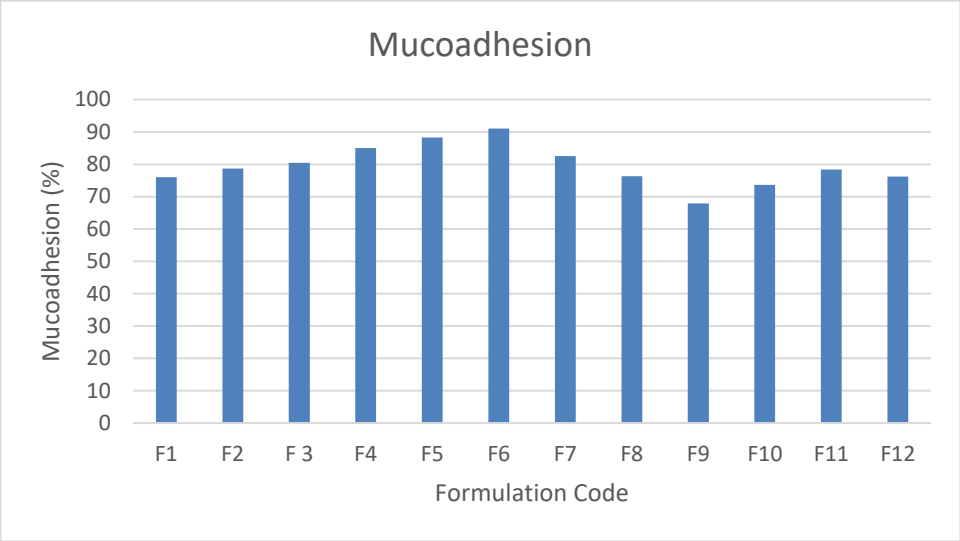

Figure 8: Mucoadhesiveness of Levofloxacin hemihydrate loaded microspheres

Mucoadhesion of Levofloxacin hemihydrate loaded microspheresranges from 67.85 % to 91.03 %. It was found that, as the amount of polymer was increased, the percentage in vitro mucoadhesion also increased. This may be due to the fact that, as the amount of polymer increased, the amino groups available for binding with the sialic acid residues in mucus layer also increase, and that results in the increase in the in vitro mucoadhesion of microspheres.

Invitro drug Release

Table 7: Invitro drug release of Levofloxacin hemihydrate loaded microspheres

T Time(hrs)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
0	0	0	0	0	0	0	0	0	0	0	0	0
1	26.37	23.52	21.24	19.21	12.83	35.9	30.17	22.73	17.52	15.36	23.43	17.45
2	47.89	39.56	35.42	29.75	21.45	53.7	47.12	43.33	39.21	34.52	42.17	26.97
3	59.48	51.55	47.13	42.54	29.13	66.5	58.25	55.85	48.35	44.63	54.62	36.54
4	68.24	60.13	56.16	50.89	35.33	77.8	65.88	61.53	56.26	51.79	63.53	45.74
5	77.65	68.89	62.93	58.89	42.11	86.6	71.23	67.17	63.31	57.85	70.14	52.75
6	86.22	76.85	72.57	66.87	50.89	97.2	76.44	72.82	66.55	63.57	79.82	60.33
7	97.52	86.6	81.94	75.87	59.9		81.19	78.56	74.46	70.83	89.14	68.77
8		97.19	87.49	86.22	69.36		86.72	83.81	81.85	76.59	98.35	78.84
9			97.56	97.83	80.22		93.21	91.31	86.52	83.85		90.61
10					93.26		97.85	96.25	94.62	91.83		99.54

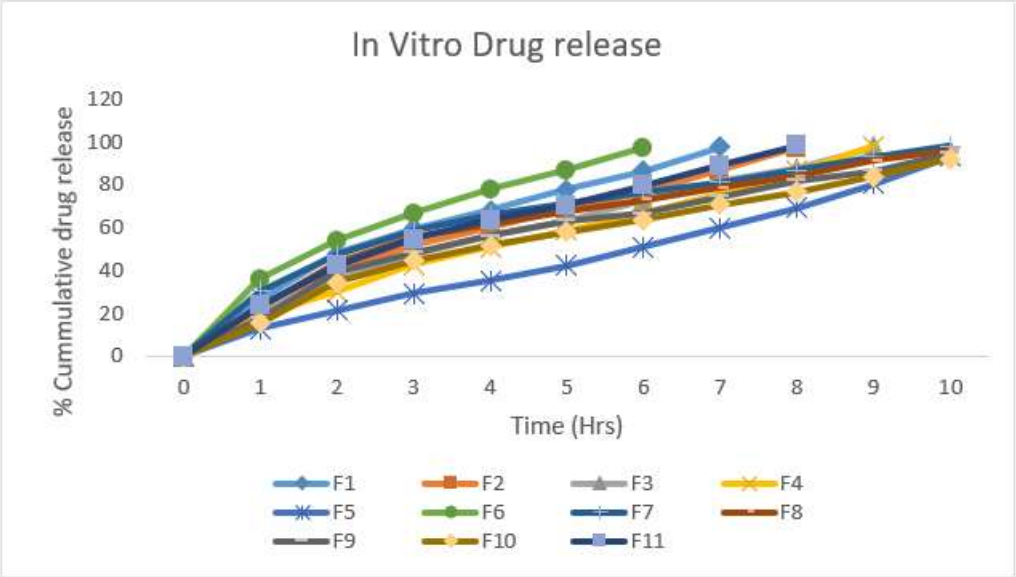

Figure 9: Invitro drug release of Levofloxacin hemihydrate loaded microspheres

The in vitro drug release profiles for all batches are shown in the above Table. The release of active agent from the matrix involves initial swelling followed by diffusion of the drug. Dissolution study of the formulated microparticles was performed in 900 ml 0.1 N HCl (pH 1.2) and drug release was found to be negligible. This was due to insolubility of drug in acidic medium. The percent drug release was as follows; F1- 97.52, F2- 97.19, F3- 97.56, F4- 97.83, F5- 93.26, F6- 97.2, F7- 97.85, F8- 96.25, F9- 94.2, F10- 91.83, F11- 98.35 and F12-99.54.It was found that more than 95 % of drug release was achieved in maximum of the formulation with different drug:polymer ratio. The reason for this drug release may be due to the proportion of the polymer ratio that increases the polymer matrix density and thus result in increased diffusional path length, leading to a decrease in drug release from the microspheres.

Kinetics of drug release

Table 8: Kinetics of drug release

CUMULATIVE (%) RELEASE Q	TIME (T)	ROOT (T)	LOG(%) RELEASE	LOG (T)	LOG (%) REMAIN	RELEASE RATE (CUMULATIVE % RELEASE / t)	1/CUM% RELEASE	PEPPAS log Q/100	% Drug Remaining	Q01/3	Qt1/3	Q01/3-Qt1/3
0	0	0			2.000				100	4.642	4.642	0.000
12.83	1	1.000	1.108	0.000	1.940	12.830	0.0779	-0.892	87.17	4.642	4.434	0.208
21.45	2	1.414	1.331	0.301	1.895	10.725	0.0466	-0.669	78.55	4.642	4.283	0.359
29.13	3	1.732	1.464	0.477	1.850	9.710	0.0343	-0.536	70.87	4.642	4.138	0.503
35.33	4	2.000	1.548	0.602	1.811	8.833	0.0283	-0.452	64.67	4.642	4.014	0.628
42.11	5	2.236	1.624	0.699	1.763	8.422	0.0237	-0.376	57.89	4.642	3.868	0.773
50.89	6	2.449	1.707	0.778	1.691	8.482	0.0197	-0.293	49.11	4.642	3.662	0.980
59.9	7	2.646	1.777	0.845	1.603	8.557	0.0167	-0.223	40.1	4.642	3.423	1.219
69.36	8	2.828	1.841	0.903	1.486	8.670	0.0144	-0.159	30.64		3.129	
80.22	9	3.000	1.904	0.954	1.296	8.913	0.0125	-0.096	19.78		2.704	
93.26	10	3.162	1.970	1.000	0.829	9.326	0.0107	-0.030	6.74		1.889	
	11	3.317		1.041	2.000	0.000		#NUM!	100		4.642	
	12	3.464		1.079								

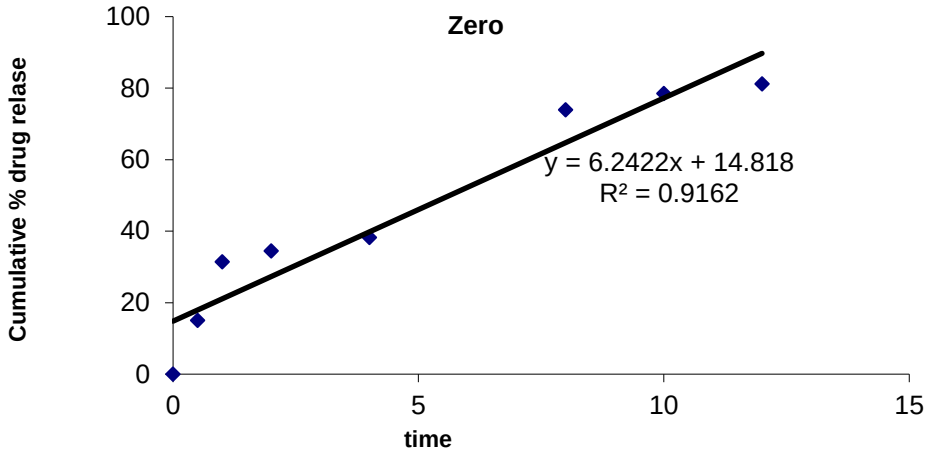

Figure 9: Zero order Kinetics of drug release

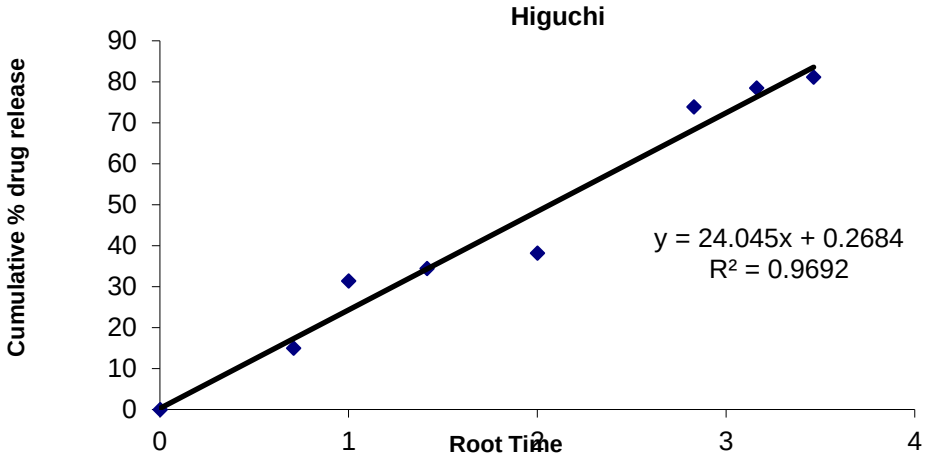

Figure 10: Higuchi Kinetics of drug release

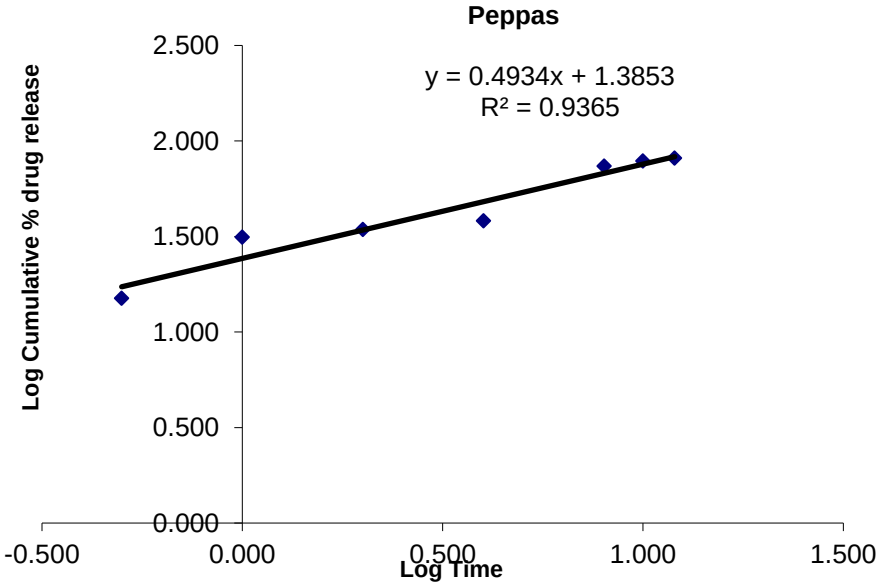

Figure 11: Peppas Kinetics of drug release

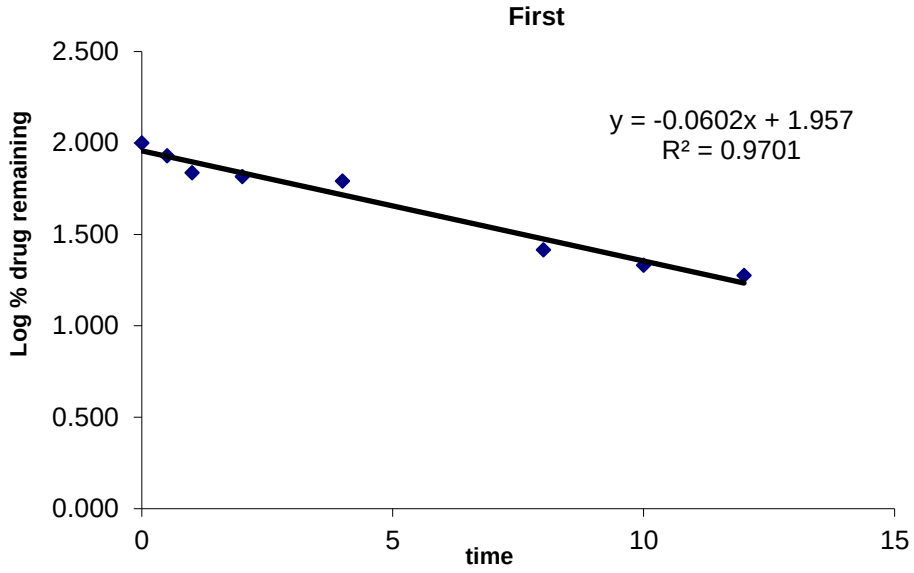

Figure 12: First order Kinetics of drug release

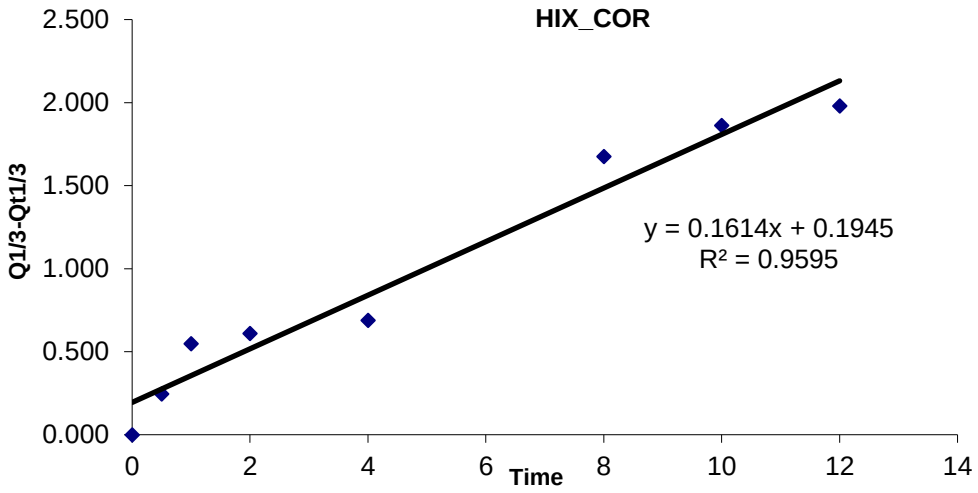

Figure 13: Hix Cor Kinetics of drug release

The interpretation of data was based on the value of the resulting regression coefficients. When the release data of levofloxacin hemihydrate loaded microspheres were plotted according to the first order equation, the formulations showed a fairly good linearity, with a R2 value of 0.9522. Whereas the same data, when plotted according to the zero order equation, improved the R2 value of 0.9. In our experiment, the in vitro release profiles of levofloxacin hemihydrate from all the formulations could at best be expressed byHiguchi's equation, as the plots showed good linearity with R2 value of 0.968. Good linearity was observed with the zero order equation. The slope of the regression line from the Higuchi plot indicates the rate of drug release and thus confirmed that the mode of release was diffusion, and to further confirm the diffusion mechanism.The results showed that when an appropriate blend of these polymers was used, the drug release became more uniform.

Accelerated stability testing according to ICHQ 1A (R2)

Table 9: Accelerated stability data of Levofloxacin hemihydrate loaded mucoadhesive beads

[Tested according to ICH Q1A(R2)]

S. No	Time (days)	Mucoadhesive strength	Drug content (%)	Drug release (%)
1	Before storage (0 day)	88.25	59.48	93.26
2	30 days (After storage*)	87.39	59.39	93.02
3	90 days (After storage*)	86.82	59.31	92.96

4	180 days (After storage*)	86.26	59.12	92.31

*Storage at 40°C and 75% RH

The purpose of study is to predict the shelf life of a product, by accelerating the rate of decomposition by increasing the temperature and relative humidity (RH).The optimized formulation were stored in a stability chamber at 40 ± 2°C and at a humidity of 75 ± 5% RH for 6 monthsandobserved for the drug content, mucoadhesiveness and in vitro drug release on 0, 30, 90, and 180 days. The zero time samples were used as controls. No remarkable changes were observed in drug content, mucoadhesiveness and in vitro drug release in stability studies.

Conclusion

The results of our present study clearly represent promising capabilities of Levofloxacin hemihydrate load mucoadhesive microspheres for orallydrug delivery as well as it could be viewed as substitute toconventional dosage form. From this study, addition of mucoadhesive polymers such as Chitosan and HMC K15M were added to prepare microspheres.Different ratios of chitosan and HPMC K15M polymer concentration were studied to explore the mucoadhesion.The polymers such as Chitosan and HMC K15M were effectively showed excellent mucoadhesive properties and controlled release property. Thus, the proposed mucoadhesive microspheres might make a contribution in complete eradication of H. pylori owing to prolonged stomach residence time and small particle size.

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