FORMULATION AND EVALUATION OF LEVOFLOXACIN HEMIHYDRATE LOADED MUCOADHESIVE ALGINATE BEADS FOR THE TREATMENT OF *HELICOBACTER PYLORI* INFECTION

A Dissertation submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY,

CHENNAI- 600 032

In partial fulfillment of the award of the degree of

MASTER OF PHARMACY

IN

Branch - I – PHARMACEUTICS

Submitted by

Name: MANIVASAKAM P

REG. No. 261710253

Under the Guidance of

Dr. R. SAMBATHKUMAR, M. Pharm., Ph.D.,

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J.K.K. NATTRAJA COLLEGE OF PHARMACY

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TAMILNADU

MAY – 2019

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CERTIFICATES

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"Formulation and Evaluation of Levofloxacin Hemihydrate Loaded Mucoadhesive Alginate Beads for the treatment of *Helicobacter Pylori* Infection",** submitted by the student bearing **Reg. No: 261710253** to "**The Tamil Nadu Dr. M.G.R. Medical University – Chennai**", in partial fulfillment for the award of Degree of **Master of Pharmacy** in **Pharmaceutics** was evaluated by us during the examination held on……..…….

Internal Examiner **External Examiner**

CERTIFICATE

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Dr. R. Sambathkumar, M. Pharm., Ph.D.,
Guide and Principal,

Dr. S. Bhama, M. Pharm., Ph.D.,
Associate Professor & HOD,
Department of Pharmaceutics

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DECLARATON

I do hereby declared that the dissertation **"Formulation and Evaluation of Levofloxacin Hemihydrate Loaded Mucoadhesive Alginate Beads for the treatment of *Helicobacter Pylori* Infection",** submitted to "**The Tamil Nadu Dr. M.G.R Medical University - Chennai",** for the partial fulfilment of the degree of **Master of Pharmacy** in **Pharmaceutics,** is a bonafide research work has been carried out by me during the academic year 2017-2018, under the guidance and supervision of **Dr. R. Sambathkumar, M. Pharm., Ph.D.,** Guide and Principal, Department of Pharmaceutics, J.K.K. Nattraja College of Pharmacy, Kumarapalayam.

I further declare that this work is original and this dissertation has not been submitted previously for the award of any other degree, diploma, associate ship and fellowship or any other similar title. The information furnished in this dissertation is genuine to the best of my knowledge.

Place: Kumarapalayam **MANIVASAKAM P**

Date: **Reg.no. 261710253**

Dedicated to Parents,

Teachers & My Family

ACKNOWLEDGEMENT

ACKNOWLEDGEMENT

First and the foremost, I am very much indebted to my respectful Guide and Principal **Dr. R. Sambathkumar, M. Pharm., PhD.,** Department of Pharmaceutics, J.K.K. Nattraja college of Pharmacy, Namakkal, Tamil Nadu, India, who constantly motivated me to do this work and helped me in every situation and his suggestions and constant support during the period of my work.

My sincere thanks and respectful regards to our reverent Chairperson **Smt. N. Sendamaraai, B.Com.,** and Director **Mr. S. Omm Sharravana, B.Com., LLB.,** J.K.K. Nattraja Educational Institutions, Kumarapalayam for their blessings, encouragement and support at all times.

It is most pleasant duty to thank for our beloved Principal **Dr. R. Sambathkumar, M.Pharm., Ph.D.,** Professor & Head, Department of Pharmaceutics, J.K.K. Nattraja College of Pharmacy, Kumarapalayam for ensuring all the facilities were made available to me for the smooth running of this project and tremendous encouragement at each and every step of this dissertation work. Without his critical advice and deep-rooted knowledge, this work would not have been a reality.

My sincere thanks to **Dr. N.S. Bhama, M.Pharm., PhD.,**Associate Professor and Head, Department of Pharmaceutics, **Mr. R. Kanagasabai, B. Pharm, M.Tech.,** Assistant Professor, **Dr. V. Kamalakannan, M.Pharm.,Ph.D.,** Associate Professor, **Mr. K. Jaganathan, M. Pharm.,** Assistant Professor, **Mr. C. Kannan, M.Pharm.,** Assistant Professor, **Ms. S. Manodhini Elakkiya, M. Pharm.,** Lecturer, **Mr. M. Subramani, M. Pharm.,** Lecturer, **Dr. Rosmi Jose, Pharm.D.,** Lecturer, Department of Pharmaceutics, for their help during my project.

My sincere thanks to **Dr. N. Venkateswaramurthy, M.Pharm., Ph.D.,** Professor and Head, Department of Pharmacy Practice, **Dr. P.Balakumar, M.Pharm., Ph.D.,** Professor, Mrs**. K. Krishna Veni, M.Pharm.,** Assistant Professor, **Mr. R. Kameswaran M.Pharm,** Assistant Professor, **Dr. Mebin Alias, Pharm.D.,** Assistant Professor, **Mrs. P. J. Sujitha,** Lecturer, **Dr. Cindy Jose, Pharm.D.,** Lecturer, **Dr. Krishna Ravi, Pharm.D.,** Lecturer, and **Dr. S.K.Sumitha, Pharm.D.,** Lecturer, Department of Pharmacy Practice, for their help during my project.

It is my privilege to express deepest sense of gratitude towards **Dr. M. Vijayabaskaran**, **M.Pharm., Ph.D.,** Professor & Head, Department of Pharmaceutical chemistry, **Mrs. S. Gomathi M.Pharm.,** Lecturer, **Mrs. B. Vasuki, M.Pharm.,** Lecturer and **Mrs. P. Devi, M.Pharm.,** Lecturer, **Mrs. P. Lekha, M.Pharm.,** Lecturer, for their valuable suggestions and inspiration.

My sincere thanks to **Dr. V. Sekar, M.Pharm., Ph.D.,** Professor & Head, Department of Analysis, **Dr. I. Caolin Nimila, M.Pharm., Ph.D.,** Assistant Professor, and **Ms. V. Devi, M.Pharm.,** Lecturer, **Mr. D. Kamalakannan, M.Pharm.,** Assistant Professor, Department of Pharmaceutical Analysis for their valuable suggestions.

My sincere thanks to **Dr. Senthilraja, M.Pharm., Ph.D.,** Associate Professor and Head, Department of Pharmacognosy, **Mrs. Meena Prabha M.Pharm.,** Assistant professor, Department of Pharmacognosy for their valuable suggestions during my project work.

My sincere thanks to **Dr. R. Shanmugasundaram, M.Pharm., Ph.D.,** Vice Principal & HOD, Department of Pharmacology, **Dr. C. Kalaiyarasi, M.Pharm., Ph.D., Mr. V. Venkateswaran, M.Pharm.,** Assistant Professor, **Mrs. M.Sudha M.Pharm.,** Assistant Professor, **Mrs. R. Elavarasi, M.Pharm.,** Lecturer, **Mrs. M. Baby kala, M.Pharm.,** Lecturer, Department of Pharmacology for their valuable suggestions during my project work.

I greatly acknowledge the help rendered by **Mrs. K. Rani,** Office Superintendent, **Miss. M. Venkateswari, M.C.A.,** typist**, Mrs. V. Gandhimathi, M.A., M.L.I.S.,** Librarian, **Mrs. S. Jayakala B.A., B.L.I.S.,** and Asst. Librarian for their co-operation. I owe my thanks to all the technical and non-technical staff members of the institute for their precious assistance and help.

Last, but nevertheless, I am thankful to my lovable parents and all my friends for their co-operation, encouragement and help extended to me throughout my project work.

MANIVASAKAM P

Reg. No. 261710253

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CHAPTER 1

Introduction

CHAPTER 2

Drug Profile

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Literature Review

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1. INTRODUCTION

Helicobacter pylori (*H. pylori)*, previously known as *Campylobacter pylori*, is a Gram-negative, microaerophilic bacterium usually found in the stomach. It is one of the common bacterial pathogens in the world, *H. pylori* infects more 50 % world's population.[1,2] *H. pylori* infection is accountable for most casesof inflammatory gastritis, peptic ulcer disease, and gastric cancerin the human population.[3] Globaly, the standard treatment of *H. pylori* infection contains two antibiotics (clarithromycin plus amoxicillin or metronidazole) and a proton pump inhibitor, called triple therapy, which remains the first line oftreatment in the clinic.[4] However, *H. pylori* eradication rates with triple therapy have significantly reduced, varying from 60to 75%, as a result of an increase in the emergence of *H. pylori* strains resistant to these antibiotics.[5] Specifically, resistance prevalence of *H. pylori* to metronidazole, which is a key component of the triple-therapy regimen, has increased to ~40% in developed countries, with an even higher prevalence of~90% in developing countries.[6,7]

Adaptation to the stomach

To avoid the acidic environment of the interior of the stomach (lumen), *H. pylori* uses its flagella to burrow into the mucus lining of the stomach to reach the epithelial cells underneath, where it is less acidic.[8] *H. pylori* is able to sense the pH gradient in the mucus and move towards the less acidic region (chemotaxis). This also keeps the bacteria from being swept away into the lumen with the bacteria's mucus environment, which is constantly moving from its site of creation at the epithelium to its dissolution at the lumen interface.[9]

H. pylori is found in the mucus, on the inner surface of the epithelium, and occasionally inside the epithelial cells themselves.[10] It adheres to the epithelial cells by producing adhesins, which bind to lipids and carbohydrates in the epithelial cell membrane. One such adhesin, BabA, binds to the Lewis b antigen displayed on the surface of stomach epithelial cells.[11] *H. pylori* adherence via BabA is acid sensitive and can be fully reversed by increased pH. It has been proposed that BabA's acid responsiveness enables adherence while also allowing an effective escape from unfavorable environment at pH that is harmful to the organism.[12] Another such adhesin, SabA, binds to increased levels of sialyl-Lewis x antigen expressed on gastric mucosa.[13]

In addition to using chemotaxis to avoid areas of low pH, *H. pylori* also neutralizes the acid in its environment by producing large amounts of urease, which breaks down the urea present in the stomach to carbon dioxide and ammonia. These react with the strong acids in the environment to produce a neutralized area around *H. pylori*.[14] Urease knockout mutants are incapable of colonization. In fact, urease expression is not only required for establishing initial colonization but also for maintaining chronic infection.[15]

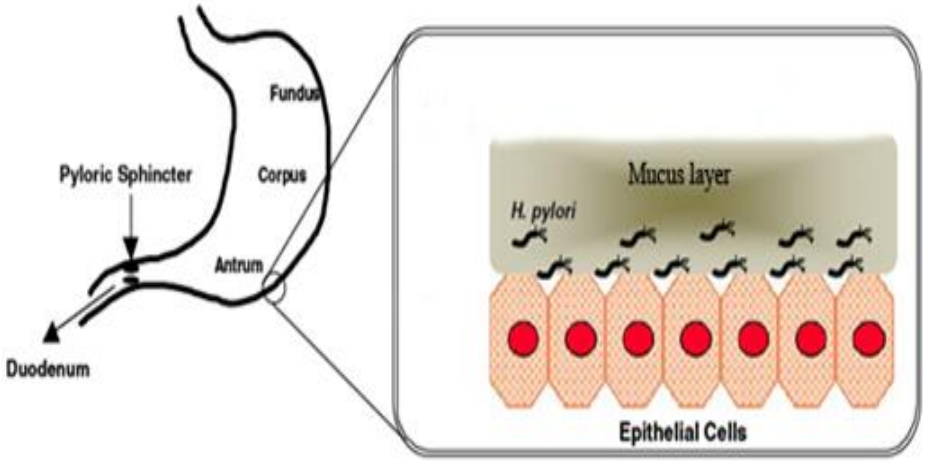


Figure 1: Schematic illustration for location of *H. pylori* within the stomach.

PREVALENCE OF *H. PYLORI* & RECENT CHANGES

Recent research has consistently shown that the prevalence of *H. pylori* is declining in the developed world and especially so in children suggesting that the infection will die out in due course. This is one reason put forward to suggest that population screen and treat may be unnecessary in these countries. However, this argument takes no account of ethnic groups, the effects of migration and those economically disadvantaged communities where infection rates are often much higher; therefore, a selective approach to screen and treat might be considered. The importance of local differences in prevalence is, therefore, important, and a number of interesting studies have been reported this year. An excellent review relating to these issues is set out by Mitchell and Katelaris.[16] A number of original studies have focused on children. One study from Iceland[17] studied 205 children aged between 7 and 18 years and found only 3.4% to be infected. However, the prevalence was 2.6% among children where both parents were born in a low prevalence country compared to 17% among those where at least one parent had been born in a high prevalence area

(P=.026). Seroprevalence in Icelandic adults is 30%-40%.

Studies from Japan have also shown a considerable fall of *H. pylori* prevalence in childhood. One study from a high GC incidence area found only 85 of 1,765 (4.8%) students aged 13-15 years to be positive,[18] and in another the prevalence in school children aged 12-15 years was 3.1%.[19] Inoue[20] reported that Japanese generations born before 1950 have a high prevalence of around 80%-90%, decreasing with age to reach around 10% or less in those born around the 1990s,and less than 2% for those born after year 2000. Similar trends are seen in China where in Hangzhou[21] the positivity rates were 14.8%, 20.2%, and 25.8% in 3-6, 7-11 and 12-17 years age groups respectively, with the overall prevalence decreasing from 21.6% to 17.2% between 2007 and 2014. In adults undergoing health checks in urban China,[22] the prevalence fell from 31.9% in the 1950-1959 birth cohort down to 20% in those born after 1990. This decrease correlated with the increase in per capita gross domestic product. The prevalence of *H. pylori* has also declined in Iran[23] where a meta-analysis estimates an overall prevalence of 54%, with a prevalence of 42% in children and 62% in adults. Initial reports of *H. pylori* infection from Iran had earlier indicated a prevalence of more than 85%. Prevalence continues to decline in Sweden.[24] In Latvia on the other hand there has been no evidence of a fall in prevalence in children over the last 10 years.[25]

H. PYLORI RELATED DISEASES

Acute infection with *H. pylori* results in histologically proven gastritis clinically manifested by epigastric fullness, vomiting, soft stools, irritability and "putrid breath" as described by Barry Marshall et al in 1985 while trying to fulfill Kochs postulates with self ingestion of live organisms.

This experiment was repeated in 1987 by Morris and Nicholson with similar results and evidence of chronic gastritis. Although spontaneous clearance may occur, the majority of the patients will develop an asymptomatic chronic state in which there is histologic evidence of gastritis with normal gastric acid production.[26]

Infection with *H. pylori* has been linked to many disease states but data support a strong association with only a few conditions, which include peptic ulcer

disease, gastric adenocarcinoma, and gastric lymphoma.[27] Other associations including the role in non-ulcer dyspepsia have yet to be confirmed.

Peptic Ulcer Disease (PUD)

H. pylori is clearly associated with both duodenal and gastric ulcers. Patients with *H. pylori* infection have been shown to have at least a threefold increased risk of developing duodenal ulcers.[28] In addition, approximately 90%-95% of patients with duodenal ulcers and 70%-90% with gastric ulcers are infected with *H. pylori*.[27,29,30] The most important evidence for a causal association between *H. pylori* and PUD is that the disease process reverses upon the eradication of the organism. Less than 10% of patients that have received an effective treatment against *H. pylori* have recurrences compared with more than 70% of those that only received acid-suppressive therapy.[31,32] The link between *H. pylori* and PUD has also been reinforced by studies done in smokers in which a twofold increase in the risk of ulcerative disease disappears after cure of *H. pylori* infection.[33] The role of *H. pylori* in gastric ulcers, although not as well studied as in duodenal ulcer disease, is similar to duodenal disease.[31]

Although the exact pathogenesis of PUD remains unclear, the following hypothesis has been proposed. *H. pylori* causes antral endocrine cells to release somato statin[34,35] which results in postprandial gastrin release. This hypergastrinemic state increases acid production and predisposes the host to develop gastric metaplasia. Gastric metaplasia is also enhanced by concomitant risk factors such as smoking, alcohol, non-steroidal anti-inflammatory drugs (NSAID) or *H. pylori* pathogenic factors such as cagA or vacA genotype. It appears that these two genetic loci are relevant to the clinical consequences of *H. pylori* infection.

Virtually every patient with PUD is infected with a agA positive strain, and vacA positivity determines the interaction with epithelial cells causing the inflammatory reaction and vacuolization reaction.

Gastric adenocarninoma

Although the incidence of gastric cancer has been declining worldwide since the 1930s, it is still one of the most common human malignancies. Evidence for an association between *H. pylori* infection and gastric cancer first came from

epidemiological studies. The prevalence of *H. pylori* infection paralleled that of gastric cancer in different populations around the world. There is a three to eightfold increase in the risk of gastric cancer in *H. pylori* infected patients. In addition, *H. pylori* infection preceded gastric cancer in other studies.[36,37,38] About half of the malignancies involving the gastric body and antrum are linked to *H. pylori* infection but tumors arising in the gastroesophageal junction are not associated with this infection.[27] Individuals with infection involving the gastric body have a higher risk than those with infection involving the antrum. These patients seem to have less dense colonization with H. pylofi and a state of hypochlorhydria as compared with patients with antral involvement.[39] On the other hand, most of the people with *H. pylori* infection will not develop gastric cancer.

A recently published prospective study from Japan that included 1526 patients followed over an average of eightyears.[40] They found a significantly higher incidence of gastric cancer in the *H. pylori* positive patients with history of nonulcer dyspepsia, gastric ulcers, and hyperplastic gastric polyps, but not among those with duodenal ulcers.

The pathogenesis of gastric cancer is believed to be different than that of PUD. It has been shown that patients with ulcerative disease actually have a lower incidence of gastric cancer.[40,41] It is known that chronic epithelial injury has a carcinogenic effect in many tissues and is thought to be one of the mechanisms implicated in the development of gastric cancer in patients infected with *H. pylori*. This organism resides in the gastric mucosa and it causes chronic superficial gastritis. Differences in bacterial virulence and a combination of host factors, such as differences in the immune and reparative responses, may determine the ultimate outcome.[42] Inflammation will induce cell proliferation, mutation and eventually selection of the fittest mutant clone.[27,43] There is also a release of free radicals that can damage DNA nucleotides which will lead to mutations and if left unrepaired can result in metaplasia and cancer.[27] Finally, in 1994 the World Health Organization declared *H. pylori* to be a type I carcinogen and a definite cause of cancer in humans.[44] The effect of *H. pylori* eradication in preventing gastric cancer is still unclear. Some studies have shown regression of preneoplastic changes in patients successfully treated for *H. pylori*,[45,46] but other studies have failed to show this association.[47,48]

Gastric lymphoma

H. pylori infection appears to lead to development of gastric lymphoid tissue that is not usually found in normal mucosa. This mucosa-associated lymphoid tissue (MALT) can undergo malignant transformation into a rare lowgrade B cell lymphoma of the stomach. This organism has been found in the majority of patients with this type of lymphoma[49] and what is even more remarkable is that 70% of patients with MALT lymphoma have shown to have a complete regression after successful treatment for *H. pylori* infection.[50] Patients with large tumors or with deep invasion into the gastric wall are less likely to respond to therapy.[48] Reinfection with *H. pylori* can cause recurrence or the tumor process.[51]

A causative role of *H. pylori* in the development of non- Hodgkins lymphoma of the stomach, the most common form of primary gastric lymphoma, has also been suggested.[52] Chronic antigenic stimulation by *H. pylori* has been proposed as the mechanism.[53]

Role in nonulcer dyspepsia

Nonulcer dyspepsia is defined as the presence of pain or discomfort in the epigastrium, associated with nausea, vomiting, heartburn, early satiety, anorexia and belching, and with no evidence of structural or biochemical abnormalities in the gastric mucosa. The annual prevalence in western countries is approximately 25%, and it accounts for about 5% of office visits.[54] A possible role of *H. pylori* in the etiology of this entity has been suspected since the organism was first linked to gastritis. However, current evidence does not seem to support this relationship. Some studies, including metaanalyses, have found a slight benefit in terms of symptomatic relief in patients who have received therapy against *H. pylori* compared with those treated only with acid suppressive therapy.[55,56] These studies have been found to have methodologic weaknesses in the definition of nonulcer dyspepsia, the regimens used, and the documentation of *H. pylori* eradication was not well documented. A recently published meta-analysis of seven randomized controlled trials, using combination therapy against *H. pylori* and with adequate follow-up to assess therapeutic response, did not find a significant trend towards a beneficial effect of therapy.[57]

Role in other diseases

H. pylori has been linked to several other clinical conditions, such as hypertrophic gastropathy, bronchiectasis, rosacea, chronic urticaria, sudden infant death syndrome and coronary artery disease.[27] Some these associations may not actually represent a causative effect of *H. pylori* and several confounding factors may be implicated.

DIAGNOSIS

Diagnostic tests for *H. pylori* infection can be divided into two categories, invasive and noninvasive methods. Invasive tests involve an upper gastrointestinal endoscopy with gastric mucosal biopsy and either rapid urease testing, histology, culture or polymerase chain reaction (PCR) tests. The noninvasive tests include antibody detection, carbon labeled urea breath tests and stool antigen detection. When determining the most appropriate test for a given situation, it is important to consider several factors including:

1) if an endoscopy is planned for any other reasons,
2) is it a follow-up test for a residual infection, and
3) prior history of gastric cancer.

Invasive diagnostic tests

Rapid urease tests

Rapid urease tests are relatively inexpensive assays based on the principle that a pH change brought on by ammonia produced by *H. pylori* urease is detected by the use of an indicator.[58] These tests are highly specific and moderately sensitive.[59,60] Several different test procedures are commercially available. CLOtest derived from Campylobacter-like organism (Ballard Medical Products, Draper, Utah) employs direct placement of urease specimen on an agar gel. A change in color from yellow to red signifies the presence of *H. pylori*. Results are obtained about 24 h after tissue placement, although most reactions can be detected within 3-4 h. This test has a sensitivity of 75% to 95% and a specificity of 75% to 100%.[61] Two biopsies are recommended to optimize the interpretation, usually one from the antrum and one from the body of the stomach. Other available tests include Pylori Tek (Serim Research Corp., Elkhart, Indiana) which uses a semipermeable membrane through

which gaseous ammonia can diffuse, accelerating the reaction to about one hour with similar sensitivity and specificity. Also available is the hpfast (GI Suppl~ Camp Hill, Pennsylvania), the newest test, in which a cell-wall detergent is added to the agar in an attempt to improve test performance but clinical evaluations have demonstrated similar results to the CLO test. The rapid urease tests are based on the presence of adequate numbers of bacteria in the specimen. The sensitivity of these tests can be adversely affected by the recent use of antibacterial agents or medications that could alter the urease activity, such as proton pump inhibitors (PPI) or bismuth compounds.[60]

ERADICATION FAILURE

The success rate of standard or first-line drug therapy which consisting of amoxicillin, clarithromycin and proton pump inhibitor, is gradually decreasing over the last decade. First-line eradication therapies most commonly used in everyday clinical practice fall considerably short of the 80% intention-to-treat (ITT) eradication rates, that are considered the minimal acceptable levels as recommended in the Maastricht guidelines.[62] The objective of *H. pylori* treatment is to achieve 100% eradication, but till date no therapy achieves 100% eradication rate. Dual, triple and quadruple drug treatment therapy failed to eradicate completely in 5 to 50% of patients.[63,64]

FACTORS RESPONSIBLE FOR FAILURE OF *H. PYLORI* ERADICATION THERAPY

Recent biopsy studies[65-69] confirms that after acquiring *H. pylori* penetrates 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.[69,70] For effective *H. pylori* eradication, antibiotics need to enter into the gastric mucus 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 the mucous layer for sufficient period of time. This is one of the main reason for failure of *H. pylori* eradication

therapy. In order to increase the eradication rate, it is essential to design suitable dosage forms to deliver the antibiotics into the site of infection.[71,72] Non compliance, bacterial resistance, cost of drugs and duration of the treatment also influences the *H. pylori* eradication.[71-75]

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.[76] It is a potentially serious problem in *H. pylori* eradication therapy. Conventional tablets and capsules are not delivering the sufficient antibiotic concentration for sufficient period of time in the mucus because of its shorter residence time in the stomach. In order to increase contact time, high doses of antibiotics are commonly prescribed, which causes adverse effects and, also it affects entire microbial flora of the gastro-intestinal tract.[77,78]

DRUG DELIVERY SYSTEMS FOR GASTRIC RETENTION

It is essential to design suitable drug delivery systems to deliver the antibiotics into the mucus layer where *H. pylori* exist. Gastric residence time of the dosage forms is important for delivery of drug into the mucus. Gastroretentive systems are commonly used to increase gastric residence time of dosage forms. Some of the gastroretentive dosage forms discussed below

a. Floating Systems[79,80]

Floating systems were mostly used to increase the gastric residence time of the dosage since 1970. Various types of floating systems have been reported, such as hollow microspheres, raft-forming systems, hydrodynamically balanced systems [HBS] and gas-generating systems. Due to the variability in gastric transit times from between person to person, floating systems were not able to produce reproducible gastric residence time, and also these systems required sufficient amount of gastric fluid to allow the systems to float.

b. Mucoadhesion

Fixing of two surfaces is called adhesion. Adhesion of natural or synthetic substances into the biological material are called “Bioadhesion”. If the biological material is mucus the term “Mucoadhesion” is commonly used.[81,82]

c. Mucoadhesive Systems

Mucoadhesive systems are adhere into the mucus layer. When the dosage forms deliver the drug at the site of action for prolonged period, usually the efficacy and bioavailability of the drug is increased.[82] Mucoadhesive systems adhere into the gastric mucus layer for prolonged period, and deliver the drug for sufficiently for longer period of time. Mucoadhesive drug delivery systems are highly suitable for the treatment of *H. pylori* infection, because it can deliver antibiotics directly into site of action.

MUCOADHESIVE DRUG DELIVERY SYSTEMS

Mucoadhesive dosage forms adhere into the mucus layer and release the drug at a controlled rate. Various theories have been proposed to explain the mechanisms involved in bioadhesion and mucoadhesion.[83,84]

a. Mucus: structure, function and composition Mucus is a viscous fluid secreted by goblet cells of the stomach. Mucus protects and hydrates the epithelial layer and also it prevents the entry of pathogens and toxic substances into the blood circulation.[85]

b. Composition of mucus

Glycoproteins, lipids, electrolytes and water are the main constituents of mucus.[86] The exact composition of mucus is given below:

1. Water: 95%
2. Glycoproteins and lipids: 0.5–5%
3. Mineral salts: 0.5–1%
4. Free proteins: 1%.

Depending on its site of secretion and certain disease conditions, the composition of the mucus may vary.[87]

c. Mucin: the glycoprotein of mucus

Glycoprotein part of the mucus is called mucin. Two forms of mucin are commonly found in mucus, such as membrane bound mucin and soluble secretory mucin.[88-90] Due to its high molecular weight and disulfide bridge, secretory mucins form viscous gels. Membrane-bound mucins contain a hydrophobic domain anchoring the molecules in the plasma membrane. In epithelial surfaces both types of mucins are found and to protect the surface.

Mucin consists of peptide core (10–30%) and oligosaccharide chains (70–80%). Both are linked linked via o-glycosidic bonds.[91-99] The mucin peptide core contains high levels of alanine, serine, glycine, threonine, proline and aromatic amino acids.[100-103]

Oligosaccharide part of the mucus consists of N-acetylglucosamine, galactose, N-acetylgalactosamine, fucose and sialic acid.[103] Mucus exhibit negative charge due to presence of sialic acid and sulfate residues.[104]

d. Thickness of the mucous layer and its turnover

The thickness of mucus layer controls the rate of drug entry into the blood circulation. The thickness of human stomach mucous layer has been reported to be 576±81 μm.[105] In general, the thickness of mucus layer varies depending on its site of secretion and, thickness which varies between 50 and 450 μm.[106,107]

Mucus is constantly released by goblet cells and adheres into the epithelial layer for specified period. Mucus is consistently removed from the epithelial layer by peristaltic forces. Turnover time of mucus has not been reported accurately, and usually it varies between 4–6 hours.[108-110]

THEORIES OF MUCOADHESION

There are four main theories that explains the possible mechanisms of mucoadhesion they are given below

1. Electronic theory
2. Adsorption theory
3. Wetting theory
4. Diffusion theory.

a. The electronic theory

According to this theory mucoadhesion occurs due to transfer of electrons between mucoadhesive polymer and mucus.[111,112]

b. The adsorption theory

According to the adsorption theory[113-117] mucoadhesion occurs due to the formation of molecular bonding between mucoadhesive polymer and mucus by van der Waals forces and hydrogen bonds.

c. The wetting theory

The wetting theory[118-122] correlates the surface tension of the mucoadhesive polymer and the mucus.

d. The diffusion theory

According to this theory, mucoadhesiveness is achieved by interpenetration of polymer chains of mucus and mucoadhesive polymers.[123-128]

In addition to above motioned theories various polymer structure related and functional groups related factors contribute to varying degrees of polymer/mucus interactions.

FACTORS AFFECTING MUCOADHESION

a. Functional group contribution

Mucoadhesiveness mainly occurs due to interpenetration of polymer chains of mucus and mucoadhesive polymers and, formation of secondary bonding between mucus and mucoadhesive polymers. Secondary non-covalent bonding forms mainly due to hydrogen bond formation between mucus and hydrophilic functional groups of the mucoadhesive polymers such as hydroxyl (OH), carboxyl (COOH), sulphate groups (SO_4H) and amide (NH_2) groups. Polymers that have above motioned functional groups form high number of hydrogen bonds with mucus, and interact more strongly with mucus.[129]

b. Degree of hydration

Optimal hydration of mucoadhesive polymers is essential for effective mucoadhesion. Hydration of the mucoadhesive polymers occurs due to combination of osmotic forces and capillary action between the mucoadhesive polymer and the mucus layer.[130] Hydration of polymer helps for relaxation of polymer chains and interpenetration of polymer chains. Excess hydration affects mucoadhesion due to the formation of a greasy mucilage.[131]

c. Polymer chain length, molecular weight and degree of cross-linking

Mucoadhesive nature of the mucoadhesive polymers varies depending upon its molecular weight. High molecular weight is necessary for effective mucoadhesion; however, polymer which has extremely long polymer chains, was unable to diffuse and interpenetrate into mucosal surfaces.[132-135]

d. pH and charge

pH value of the physiological environment also influences the mucoadhesive nature of the polymer.[136,137] Mucoadhesive nature of polyacrylic polymers are affected considerably by pH value of the physiological environment. Carboxylic groups of polyacrylic polymers are essential for mucoadhesion. At low pH, these carboxylic groups are available in unionized state and form strong hydrogen bonding with mucus. At elevated pH values, carboxylic groups ionize, unable to form hydrogen bond with mucus. Chitosan, a positively charged polymer, it forms polyelectrolyte complexes with negatively charged mucins and exhibits strong mucoadhesion at high pH value.[138]

e. Polymer concentration

Concentration of the polymer is also considerably affects the strength of mucoadhesive nature of the polymer. The optimum polymer concentration is varies depending upon the physical state of the dosage form.[139]

MUCOADHESIVE POLYMERS

The mucoadhesive polymers that are commonly used in the preparation of mucoadhesive dosage forms are commonly classified into two types.

1. First generation mucoadhesive polymers
2. Second generation mucoadhesive polymes

a. First generation mucoadhesive polymers

The first generation mucoadhesive polymers are subdivided into three categories:

(1). Anionic polymers

(2). Cationic polymers and

(3). Non-ionic polymers

- ***Anionic polymers***

For the preparation of pharmaceutical formulations, anionic polymers are most widely used, because of its low toxicity and high mucoadhesive nature. Polymers which have carboxyl and sulphate functional groups are called anionic polymers. The most widely used anionic polymer is poly(-acrylic acid) (PAA). It has excellent mucoadhesive nature, due to the formation of strong hydrogen bonding with mucus.[140]

PAA are non-toxic, non-irritant and considered safe (GRAS (Generally Recognized As Safe) status) for oral use by the FDA.[141,142]

- ***Cationic polymers***

Chitosan is the most widely used cationic polymer. Chitosan is produced by the deacetylation of chitin. Chitosan is mostly preferred because of its polysaccharide nature, biodegradability, biodegradability and less toxic nature.[143] Chitosan binds with mucus by ionic interactions mechanism. It interacts with sialic acid and sulphonic acid substructures of mucus. Moreover, the amino groups and hydroxyl also interact with mucus by hydrogen bonding.[143]

- ***Non-ionic polymers.***

Hydroxypropylmethyl cellulose (HPMC) and Methyl cellulose (MC) are commonly used nonionic polymers. Non-ionic polymers have less mucoadhesive property compared to polyelectrolytes because of its weak interactive nature with mucus.[143] Mucoadhesive property of non ionic polymers are mainly occurs due to the penetration of its polymer chains into the mucus.[144]

b. Second generation mucoadhesive polymers

- ***Lectins***

Lectins are made up of proteins and glycoproteins. It binds with carbohydrate molecules of epithelial cells reversibly. After binding with cells, the lectins can either remain present on the cell surface or get engulfed via a process of endocytosis. Because of this nature, lectins are used to target the drug. Some bacteria use lectins to fix with the cells of the host during infection. Lectins are not commonly used because of its immunogenic or anaphylaxis nature.[145,146]

- ***Bacterial adhesions***

K99-fimbriae, an attachment lectin, obtained from *E. coli* is most widely used to target the drug into gastrointestinal tract, and also it covalently attaches with polyacrylic acids.[147]

Recently, a new types of mucoadhesive polymer has been introduced into the market. These new types of mucoadhesive polymers are prepared by introducing thiol groups into the polymeric backbone of established mucoadhesive polymers. Thiol groups interact strongly with cysteine rich port of mucus by forming disulfide bonds.[147] These disulfide bonds are not affected by ionic strength and pH of the physiological environment.

Example of thiolated polymers.[148]

Poly(acrylic acid)–homocysteine

Chitosan–iminothiolane

Poly(methacrylic acid)–cysteine

Chitosan–thioethylamidine

Poly(acrylic acid)–cysteine

Chitosan–thioglycolic acid

Sodium carboxymethylcellulose–cysteine

Poly(acrylic acid)–homocysteine

Alginate–cysteine

MUCOADHESIVE SYSTEMS IN ORAL DRUG DELIVERY

Oral mucoadhesive drug delivery systems extend the residence time of dosage forms in gastric or small intestine. Mucoadhesive systems, commonly used to deliver the drug into the site of action, target the drug into certain parts of GI tract and prolong the drug delivery.

A number of mucoadhesive dosage forms, including nanoparticles, semisolid dosage forms, microspheres, powders, sustained release tablets have been widely reported.

a. Mucoadhesive microspheres

Microsphere plays an important role in particulate drug delivery systems, because of its size and its good carrier property. One of the main drawbacks of microspheres is by its shorter gastric residence nature. These drawbacks have now been solved by coupling the mucoadhesive property to the conventional microspheres, by preparing novel "Mucoadhesive beads."

Mucoadhesive beads are commonly prepared by using mucoadhesive polymers or coating of conventional beads with mucoadhesive polymers. The size of the mucoadhesive polymers commonly varies between 1–1000 µm.[149] Mucoadhesive microspheres can be tailored to stay to any mucosal tissue including those found in urinary tract, GI tract, nasal cavity and eye.

2. DRUG PROFILE[150]

Levofloxacin hemihydrate

Generic and additional names Levofloxacin hemihydrate

Synonyms BAY 12-8039

Molecular formula $C_{18}H_{20}FN_3O_4$, ½ H_2O

Molecular weight 370.4

Description LFX is a yellowish white to yellow powder

Solubility Freely soluble in glacial acetic acid, chloroform; sparingly soluble in water

Melting point 214- 216ºC

Category Anti-Bacterial Agents, Anti-Infective Agents, Quinolones

Phamacokinetics

- Absorption LFX is rapidly and entirely absorbed after oral dose.
- bioavailability 99%
- Protein Binding 24 – 38 %
- Excretion Urinary
- Plasma Half Life 6 to 8 hr

Mechanism of action

LFX is L form of the racemate, OFX, a quinolone antimicrobial agent. The antibacterial activity of OFX resides primarily in L-isomer. The MOA of LFX involves destroying of bacterial topoisomerase and di-nucleotide adenosine gyrase enzymes required for di-nucleotide adenosine replication, transcription, repair and recombination. LFX exhibits in vitro MIC of two mcg/mL or less against most (•90%) strains

Dose (*H. pylori* infection)

Levofloxacin 500 mg b.d

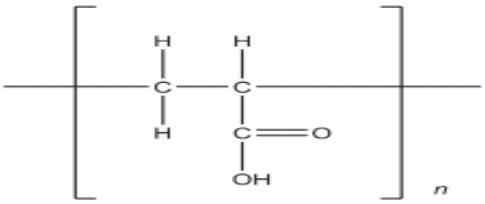
3. POLYMER PROFILE

CARBOPOL 974P[151]

Nonproprietary Names: Carbopola, Carbopols.

Synonyms: Poly acrylic acid polymer, carbopol, acrylic acid, carboxyvinyl polymer, Acritamer, carboxy poly methylene.

Structural Formula



The polymer chains of carbopols are crosslinked with allyl pentaerythritol and polymerized in ethyl acetate.

Description: Carbopols are white-colored, hygroscopic, fluffy powders

Molecular Weight: 12000-140000

Melting point: Decomposition occurs at 260ºC.

Glass transition temperature: 100 –105°C.

Moisture content: Normal content is up to 2%.

Solubility: Carbopol 974p insoluble in water, dilute acids, and common organic solvents.

Stability: Stable though hygroscopic and can be heated at temperatures below 104ºC up to 2 hours without affecting their thickening efficiency.

Safety: Carbopols are generally nontoxic and nonirritant. There is no hypersensitivity reactions are reported.

SODIUM ALGINATE [152]

Synonyms

Algin; alginic acid, sodium salt; E401; Kelcosol; Keltone;Protanal; sodium polymannuronate

Empirical Formula and Molecular Weight

Sodium alginate consists chiefly of the sodium salt of alginic acid, which is a mixture of polyuronic acids composed of residues of D-mannuronic acid and L-guluronic acid.

Typical Properties

Acidity/alkalinity: pH 7.2 for a 1% w/v aqueous solution.

Solubility: practically insoluble in ethanol (95%), ether, chloroform, and ethanol/water mixtures in which the ethanol content is greater than 30%. Also, practically insoluble in aueous acidic solutions in which the pH is less than 3. Slowly soluble in water, forming a viscous colloidal solution.

Viscosity (dynamic): various grades of sodium alginate are commercially available that yield aqueous solutions of varying viscosity. Typically, a 1% w/v aqueous solution, at 208C, will have a viscosity of 20–400 mPa s (20–400 cP).Viscosity may vary depending upon concentration, pH, temperature, or the presence of metal ions.

Incompatibilities

Sodium alginate is incompatible with acridine derivatives, crystal violet, phenylmercuric acetate and nitrate, calcium salts,heavy metals, and ethanol in concentrations greater than 5%.Low concentrations of electrolytes cause an increase in viscosity but high electrolyte concentrations cause salting-out of sodium alginate; salting-out occurs if more than 4% of sodium chloride is present.

4. LITERATURE REVIEW

Umamaheshwari et al., (2002)[153] prepared acetohydroxamic acid entrapped floating mucoadhesive microspheres by emulsion solvent diffusion technique. A 2% (w/v) solution of polycarbophil was used to prepare floating mucoadhesive microspheres. In vitro floating studies, detachment force and in vivo studies were confirmed the potential of these microspheres.

Umamaheshwari et al., (2003)[154] developed acetohydroxamic acid loaded polycarbonate microballoons by a solvent evaporation method. In simulated gastric fluid In vitro release studies were conducted. About 74% to 85% of microballoons were floated up to 12 h. In vitro cell growth studies were conducted by using *H. pylori* culture and in vivo studies were conducted by using *H. pylori* infected Mongolian gerbils. Prepared microballoons demonstrated 10 times higher anti-*H. pylori* action when compared to plain acetohydroxamic acid solution.

Hejazi and Amiji (2003)[155] developed tetracycline loaded chitosan microspheres for *H. pylori* infection. Suspensions of prepared microspheres were given to gerbils. Gerbils were killed at different time intervals to assess the radioactivity in gastric fluids and tissues. 11% of chitosan microspheres remained in the stomach after 10 hours of administration. Higher tetracycline concentration was observed in the stomach than plain drug solution and non crosslinked microspheres.

Schicho Higo et al., (2004)[156] developed tetracycline loaded sucralfate acidic complex for eradication of *H. pylori*. In vitro results confirmed that more amount of tetracycline loaded sucralfate acidic complex retained on the gastric mucosa than physical mixture of tetracycline and sucralfate. Addition of acid during the formulation dissociated the aluminium hydroxide groups from the binding sites and produced more binding sites.

Amiji (2004)[157] developed tetracycline loaded chitosan microspheres. Efficacy of prepared microspheres was evaluated by using the fasted Mongolian gerbils. Tetracycline loaded chitosan microspheres were given to *H. pylori* infection induced gerbils. A considerable increase in *H. pylori* eradication activity was observed in comparison to aqueous solution of the drug.

Ishak et al., (2007)[158] prepared metronidazole beads using chitosan and alginate by ionotropic gelation method. Prepared beads showed optimum drug entrapment efficiency, immediate buoyancy, and extensive drug release profile. In vivo *H. pylori*

studies showed that metronidazole (dose 15 mg/kg) floating beads provided 100% *H. pylori* clearance while the metronidazole suspension (dose 20 mg/kg) provided only 33.33% clearance.

Rajinikanth et al., (2008)[159] developed floating gel system of acetohydroxamic acid. Prepared floating systems formed gel immediately and floated for longer period time in simulated gastric fluid (pH 1.2). In vivo studies confirmed the anti *H. pylori* activity of floating gel system in gerbil model. Authors concluded that the quantity of acetohydroxamic acid required for *H. pylori* eradication effect was very less in floating gel system than acetohydroxamic acid suspension.

Tan S et al., (2009)[160] Real-time electronic speckle pattern interferometry method has been applied to study the diffusion behavior of levofloxacin mesylate (MSALVFX) in agarose hydrogel. The results show that the diffusivity of solute decreases with the increase of concentration of agarose and adapts to Kohlrausch's law. Furthermore, Amsden's model, based on the retardance effect associated with polymer chain flexibility, was employed to simulate the diffusion behavior. The consistent results suggest that the retardance effect dominates the diffusion process of MSALFVX in hydrogel; moreover, polymer chain flexibility greatly affects drug transport within the polymer matrix.

Chang et al., (2010)[161] prepared berberine nanoparticles for the eradication of *H. pylori*. Chitosan was used to prepare nanoparticles. The effect of the nanoparticles and their mechanisms were evaluated by using human gastric carcinoma epithelial cell line. The prepared nanoparticles significantly suppressed the *H. pylori* growth and reduced cytotoxic effects of *H. pylori*.

Arora and Budhiraja (2011)[162] prepared floating metronidazole tablets for *H. pylori* eradication. Tablets were prepared by using carbopol 971P and methocel K100LV. The floatability and drug release increased in the presence of sodium bicarbonate, microcrystalline cellulose and sodium citrate. The optimized formulation provided drug release up to 12 hours by anomalous diffusion mechanism.

Vasilev K et al., (2011)[163] prepared plasma polymerization of n-heptylamine for the generation of two thin coated layers that serve two distinct purposes. First, an n-heptylamine plasma polymer layer is applied onto the surface of the solid carrier material in order to facilitate spreading of the drug, which is applied by solvent casting; levofloxacin in ethanol was used for this study. A second n-heptylamine

plasma polymer coating then serves as a thin barrier coating to control the release. We show that the rate of release can be adjusted via the thickness of the plasma polymer overlayer. We also show that this modality of controlled release of levofloxacin completely inhibits Methicillin-resistant Staphylococcus aureus (MRSA) colonization and biofilm formation on and near the coated biomaterial surface.

Kumar G et al.,(2012)[164] prepared novel poly(lactic-co-glycolic acid) (PLGA)-based nanoformulation of levofloxacin for multidrug-resistant tuberculosis with the purpose of achieving sustained release in plasma. After lyophilization of levofloxacin-loaded nanoparticles, the average size, charge, and polydispersity index were 268 ± 18 nm, -10.2 ± 1.5 mV, and 0.15 ± 0.03, respectively. The maximum drug encapsulation efficiency and loading capacity were 36.9 ± 6.1% (w/w) and 7.2 ± 1.2 mg/100 mg nanopowder, respectively. Biphasic extended-release profile was produced in vitro. Scanning electron microscopy and Fourier transform infrared studies showed spherical shape of drug-loaded nanoparticles and no drug-polymer interactions were observed. After single oral administration in mice, levofloxacin-loaded PLGA nanoparticles produced sustained release of levofloxacin for 4 days in plasma against 24 h for free levofloxacin. Levofloxacin was detected in organs (lung, liver, and spleen) for up to 4-6 days in case of levofloxacin-loaded nanoparticles, whereas free levofloxacin was cleared within 24 h. This novel formulation did not show any significant adverse effects on body weight and clinical signs in mice. No treatment-related changes were found in hematological and biochemical parameters and on histopathological evaluation. These results indicate the feasibility of development of an orally efficacious safe formulation of levofloxacin with sustained-release properties.

El-Zahaby SA et al., (2014)[165] prepared gastroretentive levofloxacin (LVF) floating mini-tablets for the eradication of Helicobacter pylori (*H. pylori*). They were prepared using the matrix forming polymer hydroxypropyl methylcellulose (HPMC K100M), alone or with Carbopol 940P in different ratios by wet granulation technique. Buoyancy of mini-tablets was achieved by an addition of an effervescent mixture consisting of sodium bicarbonate and anhydrous citric acid to some formulations. The prepared mini-tablets were evaluated for weight variation, thickness, friability, hardness, drug content, in vitro buoyancy, water uptake and in vitro release. The optimized formula was subjected to further studies: FT-IR, DSC analysis and in vivo

examination in healthy volunteers. The prepared mini-tablets exhibited satisfactory physicochemical characteristics. Incorporation of gas-generating agent improved the floating parameters. HPMC K100M mini-tablet formulation (F1) offered the best controlled drug release (>8 h) along with floating lag time <1 s and total floating time >24 h. The obtained DSC thermograms and FT-IR charts indicated that there is no positive evidence for the interaction between LVF and ingredients of the optimized formula. The in vivo test confirmed the success of the optimized formula F1 in being retained in the stomach of the volunteers for more than 4 h. LVF floating mini-tablets based on HPMC K100M is a promising formulation for eradication of *H. pylori*.

El-Zahaby SA et al., (2014)[166] prepared size increasing (plug-type) levofloxacin hemihydrate (LVF) tablets for eradication of Helicobacter pylori (*H. pylori*). They were prepared using in situ gel forming polymers including: gellan gum, sodium alginate, pectin and xanthan gum. Effect of cross-linkers: calcium and aluminum chloride, on the drug release was also studied. The prepared tablets were evaluated for their physicochemical parameters: weight variation, thickness, friability, hardness, drug content, water uptake and in vitro drug release. The optimized formula was subjected to further studies such as radial swelling test, FT-IR and DSC. Results revealed that LVF release depends not only on the nature of the matrix but also on the type of cross linker used to form this polymeric matrix. The addition of either calcium chloride or aluminum chloride, as cross-linkers, to gellan gum formulations significantly decreased drug release. Other polymers' formulations resulted in increased drug release upon addition of the same cross-linkers. The formula containing xanthan gum without any cross linker showed the most sustained LVF release with an increase in diameter with time, thus acting as a plug-type dosage form. IR spectra and DSC thermograms of LVF, xanthan gum, and a physical mixture of both, indicated that there was no interaction between the drug and the polymer and confirmed the drug stability.

Merchant Z et al., (2014)[167] prepared powders by spray-drying from an aqueous solution containing levofloxacin and chitosan/amphiphilic octanoyl chitosan. l-leucine was also used to assess its effect on aerosolization. Following spray-drying, the resultant powders were characterized using scanning electron microscopy, laser diffraction, dynamic light scattering, HPLC, differential scanning calorimetry, thermogravimetric analysis and X-ray powder diffraction. The in vitro aerosolization

profile was determined using a Next Generation Impactor, whilst in vitro antimicrobial assessment was performed using MIC assay. Microparticles of chitosan have the property of mucoadhesion leading to potential increased residence time in the pulmonary mucus, making it important to test the toxicity of these formulations. In-vitro cytotoxicity evaluation using MTT assay was performed on A549 cell line to determine the toxicity of formulations and hence feasibility of use. The MTT assay confirmed that the polymers and the formulations were non-cytotoxic. Hydrophobically modifying chitosan showed significantly lower MIC (4-fold) than the commercial chitosan against P. aeruginosa. The powders generated were of suitable aerodynamic size for inhalation having a mass median aerodynamic diameter less than 4.5μm for formulations containing octanoyl chitosan. These highly dispersible powders have minimal moisture adsorption and hence an emitted dose of more than 90% and a fine particle fraction (FPF) of 52%. Powders with non-modified chitosan showed lower dispersibility, with an emitted dose of 72% and FPF of 20%, as a result of high moisture adsorption onto the chitosan matrix leading to cohesiveness and subsequently decreased dispersibility.

Jalvandi J et al., (2017)[168] prepared a range of biodegradable drug-nanofibres composite mats drug delivery systems. The results showed that controlled release of levofloxacin (LVF) could be achieved by covalently binding LVF to low molecular weight chitosan (CS) via a cleavable amide bond and then blending the conjugated CS with polyvinyl alcohol (PVA) nanofibres prior to electrospinning. PVA/LVF and PVA-CS/LVF nanofibres were fabricated as controls. The conjugated CS-LVF was characterized by FTIR, DSC, TGA and 1H NMR. Scanning electron microscopy (SEM) showed that the blended CS-PVA nanofibres had a reduced fibre diameter compared to the controls. Drug release profiles showed that burst release was decreased from 90% in the control PVA/LVF electrospun mats to 27% in the PVA/conjugated CS-LVF mats after 8h in phosphate buffer at 37°C. This slower release is due to the cleavable bond between LVF and CS that slowly hydrolysed over time at neutral pH. The results indicate that conjugation of the drug to the polymer backbone is an effective way of minimizing burst release behaviour and achieving sustained release of the drug, LVF.

Zhang LP et al., (2018)[169] prepared liquid crystalline molecularly imprinted polymers (LC-MIPs) by low cross-linking. The multiwalled carbon nanotubes

(MWCNTs) coated LC-MIP (MWCNT@LC-MIP) was the first fabricated as a novel floating interaction-controlled DDS. The synthesis was achieved by adding 9-vinylanthracene to obtain the high-density vinyl group functionalized MWCNTs firstly, and then polymerization of LC MIPs was performed on the surface of MWCNTs using a mixture of methacrylic acid, ethylene glycol dimethacrylate, and 4-methyl phenyl dicyclohexyl ethylene (LC monomer) with levofloxacin (LVF) as model template drug. Both template/functional monomer ratio and levels of crosslinker were optimized to obtain the best imprinting factor. Characterizations of polymer were investigated by the transmission electron microscope, nitrogen adsorption, thermogravimetric analysis. The release profiles showed an obvious zero-order release of LVF from MWCNT@LC-MIP, which exhibited 3.8 μg/h of the release rate with duration of about 20 h. In vivo pharmacokinetic study displayed the relative bioavailability of the gastro-floating MWCNT@LC-MIP was 578.9%, whereas only 58.0% of MWCNT@MIP and 11.7% of the bared MWCNT. As a conclusion, MWCNT@LC-MIP showed potentials for oral administration by the innovative combination of floating and controlled release properties.

5. MATERIALS AND INSTRUMENTS

Table 1: Materials

S.No	Materials	Supplier
1	Levofloxacin hemihydrate	Goldsun Pharmaceuticals limited, Mumbai.
2	Carbopol 974P	Macleods Pharmaceuticals limited, Mumbai.
3	Sodium Alginate	Nice Chemical, Bangalore.
4	Calicum Chloride	Nice Chemical, Bangalore.
5	Hydrochloric acid	Qualigens fine chemicals, Mumbai.
6	mucin (type II) from porcine,	Aldrich Co.
7	Iodomethane	Aldrich Co.
8	N-methyl pyrrolidone	Aldrich Co.
9	Basic fuchsin (pararosaniline),	Aldrich Co.
10	Sodium metabisulphite,	Nice Chemical, Bangalore.
11	Periodic acid,	Aldrich Co.
All other reagents were of analytical grade and used as received.		

Table 2: Instruments and apparatus

S.No	Name of the Instrument	Name of the Manufacturer and Model
1	Differential scanning calorimeter	Universal Instruments. (Model: V4.2E TA)
2	Digital balance	Shimadzu Scientific Instruments. (Model: BL-220H)
3	Dissolution test apparatus	Labindia. (Model: Disso 2000)
4	FTIR Spectrophotometer	Shimadzu, Japan. (Model: FTIR-84005)
5	Glass wares	Sigma Scientific Glass Pvt. Ltd,Chennai.
6	Laboratory shaker/vibrator	Xi'an Depai Biotechnology Co., Ltd.(Model: BILON-COS-100B)
7	Magnetic stirrer	REMI Laboratory Instruments. (Model: 2 MLH).
8	Mechanical stirrer with Digital rpm display	REMI Laboratory Instruments. (Model: RQ-121/D).
9	Particle size Analyzer	Particle sizing systems, Inc, Santa Barbara, Calif., USA (Model: 780 AccuSizer)
10	Scanning Electron Microscope	Hitachi High-Technologies. (Model: S – 450).
11	Stability chamber	REMI Laboratory Instruments. (Model: Remi CHM- 10 S®)
12	Tablet disintegration test machine (I.P. STD. 1985)	Scientechinstruments. Delhi.
13	UV/Visible double beam Spectrophotometer	Shimadzu Analytical (India) Pvt. Ltd.
14	Vacuum dryer	Saga Engineering Co (Model: SO-150)
15	Vortex mixer	Alfa Medical instruments (Model: RX3 Vortex mixer)

6. NEED OF THE STUDY

Helicobacter pylori (H. pylori) causes chronic gastritis, peptic ulcer, gastric cancer and gastric MALT lymphoma. Guidelines support treatment irrespective of symptoms and complications. Success rates of empirical therapies have fallen in recent years in many countries.The "key" antibiotics in the treatment of *H. pylori* infection are clarithromycin and levofloxacin. After failure of an empirical first-line treatment, physicians use a levofloxacin triple therapy (PPI + levofloxacin + amoxicillin) or a bismuth quadruple therapy.[170] Inparticular, levofloxacin triple therapy is the treatment of choice after failure of bismuth quadruple therapy. Even though, treatment fails to eradicate *H. pylori* infection completely. The main reasons given for the treatment failure is the short residence time of antimicrobial agents in the stomach and availability of insufficient antimicrobial concentration in the mucus layer of the stomach where *H. pylori* resides, emergence of antibiotic-resistant strains and poor adherence possible due to complicated regimens and drug-related side-effects.

It is therefore, essential to design suitable dosage forms that not only solve the limitations of conventional delivery systems but also deliver the antibiotics to the site of action. To improve treatment of *H. pylori* infection, by achieving required bactericidal concentrations of antibiotics in the stomach, it is assumed that the novel formulations adhering to the mucus layer and releasing the drug at the site of infection would be significantly more effective than conventional dosage forms. Mucoadhesive drug delivery systems may prolong the gastric residence time of the antibiotics because they adhere to the mucus and also deliver the antibiotics directly into the mucus where *H. pylori* exists. Among the mucoadhesive drug carriers, mucoadhesive beads have some advantages because of its close contact with the mucus, lightweight and smaller dose variation. Hence, in this study, mucoadhesive microsphere drug delivery system was selected to deliver levofloxacin effectively into the mucus.

To achieve the above therapeutic needs effectively, the drug delivery system should have mucoadhesive and extended release property. To achieve the mucoadhesive and extended release property in this study, combination of mucoadhesive polymers, such as sodium alginate and carbopol 974P were used.

The use of natural polymers for the design of drug delivery systems has long been the subject of great interest during the past decades. Sodium alginate is a sodium salt of alginic acid, a naturally occurring nontoxic polysaccharide found in marine brown algae. Alginate has been widely used as food and pharmaceutical additives, such as tablet disintegrant, thickening, and suspending agent. It contains two uronic acids, α-L-guluronic (G) and β-D-mannuronic acids (M) and is composed of homopolymeric blocks and blocks with an alternating sequence. This polymer can form a reticulated structure when it contacts with Ca2+ and thus it has been used to produce sustained release particulate systems for various drugs, proteins, and even cells. Gelation occurs by an ionic interaction between the calcium ions and the carboxylate anions of G-G blocks as calcium ions diffuse from the external source into the droplet forming a polyanionic microcapsule. Quick breakdown of beads in the *in vitro* release process is the main disadvantage of Ca-sodium alginate beads. Drug release from calcium-alginate beads depends on the swelling of the beads and the diffusion of the drug in the gel matrix. Alginate beads do not swell appreciably in stomach acidic fluid. It is the usual practice to use an additional secondary polymer when a primary polymer fails to provide the desired extended period of release and mucoadhesivity. To improve mucoadhesive property and modify the drug release, in this study Carbopol 974P was incorporated along with sodium alginate.

7. AIM AND OBJECTIVES OF THE WORK

Aim

The aim of the present research work has been designed to develop levofloxacin hemihydrate loaded alginate mucoadhesive beads of for the treatment of *H. pylori* infection.

Objectives of the present work was to

1. Findout the suitable concentration of sodium alginate and carbopl 974P and calcium chloride for formulating levofloxacin hemihydrate beads.
2. To charatarize the formulated levofloxacin hemihydrate beads.
3. Prolong the delivery of the antibiotics at the site of infection.
4. Minimize the dosing frequency.
5. Reduce the amount of drug required for *H. pylori* eradication.

8. METHODOLOGY

EXPERIMENTAL INVESTIGATIONS

PREFORMULATION STUDIES

Standard curve of Levofloxacin hemihydrate in 0.1 N HCl

Stock solution of Levofloxacin hemihydrate (100 µg/ml) was prepared in 0.1 N HCl, repeated three consecutive days and each day in triplicate to find the inter- and intra-day variations. It was further diluted to obtain the known standard solutions in range of 1-10 µg/ml. Absorbance was measured spectrophotometrically (Shimadzu UV/Visible spectrophotometer 2100; Tokyo, Japan) at 293 nm. The mean data (n=9) were used for the preparation of calibration curve. The concentration of the dissolved drug was calculated from regression equation obtained from calibration curve.

Preparation of sodium alginate-974P carbopol beads

Levofloxacin hemihydrate beads was formulated by using ion gelation technique. Different concentrations of sodium alginate (1 w/v%, 2 w/v% and 3 w/v %) solutions were prepared using 10 ml purified water as a vehicle. In the resuting solution, Carbopol 974P(1% w/v) was dispered and agitatated using sigma blade mixer for 5min to prevent aggregation. In the above dispersion powdered levofloxacin hemihydrate was dispersed and agitatated using sigma blade mixer for 5min to ensure uniform dispersion of levofloxacin hemihydrate. The resulting dispersion solution was added manually dropwise in a different concentrations of 40 ml calcium chloride solution (2w/v, 3w/v and 4 %w/v) through a syringe (no. 20). The added droplets were retained in the calcium chloride solution for 1 hour to complete the reaction and to produce spherical rigid mucoadhesive beads. The mucoadhesive beads were collected by decantation and the products were separately washed frequently and dried at 40°C for 3 hours in a hot air oven.

Table 3 : Composition of Levofloxacin hemihydrate loaded calcium alginate beads prepared by ion gelation method

Formulation Code	Sodium Alginate (% w/v)	Carbopol 974P (% w/v)	Calcium chloride (% w/v)	Levofloxacin hemihydrate (% w/v)
LM1	1	1	2	2.5
LM 2	2	1	2	2.5
LM 3	3	1	2	2.5
LM 4	1	1	3	2.5
LM 5	2	1	3	2.5
LM 6	3	1	3	2.5
LM 7	1	1	4	2.5
LM 8	2	1	4	2.5
LM 9	3	1	4	2.5

EVALUATION OF MUCOADHESIVE BEADS

Determination of percentage yield of beads[171]

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

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

Determination of drug content and encapsulation efficiency[172]

The drug content of the beads were measured by extraction method. Accurately weighed 5 mg of mucoadhesive beads were crushed in to a powder using glass mortar and pestle. The crushed beads 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 ± 0.5ºC. The samples were then filtered to obtained clear solution and analyzed for the drug content HPLC.

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

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

Particle size analysis

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

Shape and surface characterization

The shape and surface characteristics of the beads were observed under a Scanning Electron Microscope (SEM). HITACHI-SEM MODEL S – 450 model scanning electron microscope was used for the study. The prepared beads 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[173]

A periodic acid/Schiff (PAS) colorimetric method reported by Mantle and Allen174 was used to determine the free mucin concentration in order to assess the amount of mucin adsorbed on the levofloxacin hemihydrate mucoadhesive beads and its effect on the assessment of mucoadhesive behavior of prepared mucoadhesive beads. 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 curves 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 a UV spectrophotometer.

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 beads was also assessed with the above procedure. Each experiment was performed 3 times and standard deviation noted.

Adsorption of Mucin on Alginate Beads

Mucin aqueous solution with different concentrations (0.025, 0.05, 0.1, 0.2, and 0.5 mg/mL) were prepared. Levofloxacin hemihydrate mucoadhesive beads (20 mg) were dispersed in the above mucin solutions, vortexed and shaken at room temperature.[175] 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 (1) or Langmuir (2) equations describing the adsorption isotherms:

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

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

Where C_{ads} is the concentration of mucin adsorbed at equilibrium and C_e 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, $1/C_{ads}$ was plotted against $1/C_{free}$ to get the constants and for the Freundlich equation, log C_{ads} was plotted against C_{free} to get the constants.

The mucin adsorption is estimated using the Equation

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

Compatibility studies

Fourier-Transform Infrared Spectrophotometry (FTIR)

Infrared red spectra for pure Levofloxacin hemihydrate, blank beads, levofloxacin hemihydrate mucoadhesive beads 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. The scanning range was set into 450–4000 cm^{-1}.

Differential scanning calorimeter (DSC)

The thermal analysis of pure drug, formulations and blank beads were carried out using 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 10–300ºC was scanned using a heating rate of 10ºC min^{-1}. A nitrogen purge of 50ml min^{-1} was used in the oven.

In vitro dissolution studies

In vitro drug release from mucoadhesive beads was analyzed by using USP dissolution test apparatus 2 (Paddle) with stirrer at 100 rpm (Disso 2000, Labindia). Predetermined quantities of beads 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 37ºC±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)

The optimized formulation (LM 6) were stored in a stability 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 release 0, 30, 90, and 180 days. The zero time samples were used as controls.

Statistical analysis

The data obtained from the production yield, encapsulation efficiency, particle size, *invitro* release studies and *in vivo* studies of beads were analyzed statistically by one-way ANOVA using GraphPad Prism software (GraphPad Software) and $P < 0.05$ was considered statistically significant.

9. RESULTS

Table 4: Standard curve of Levofloxacin hemihydrate in 0.1 N HCl

S.No	Concentration (µgm/ml)	Absorbance
1	0	0
2	1	0.013
3	2	0.023
4	3	0.033
5	4	0.046
6	5	0.050
7	6	0.064
8	7	0.073
9	8	0.085
10	9	0.093
11	10	0.114

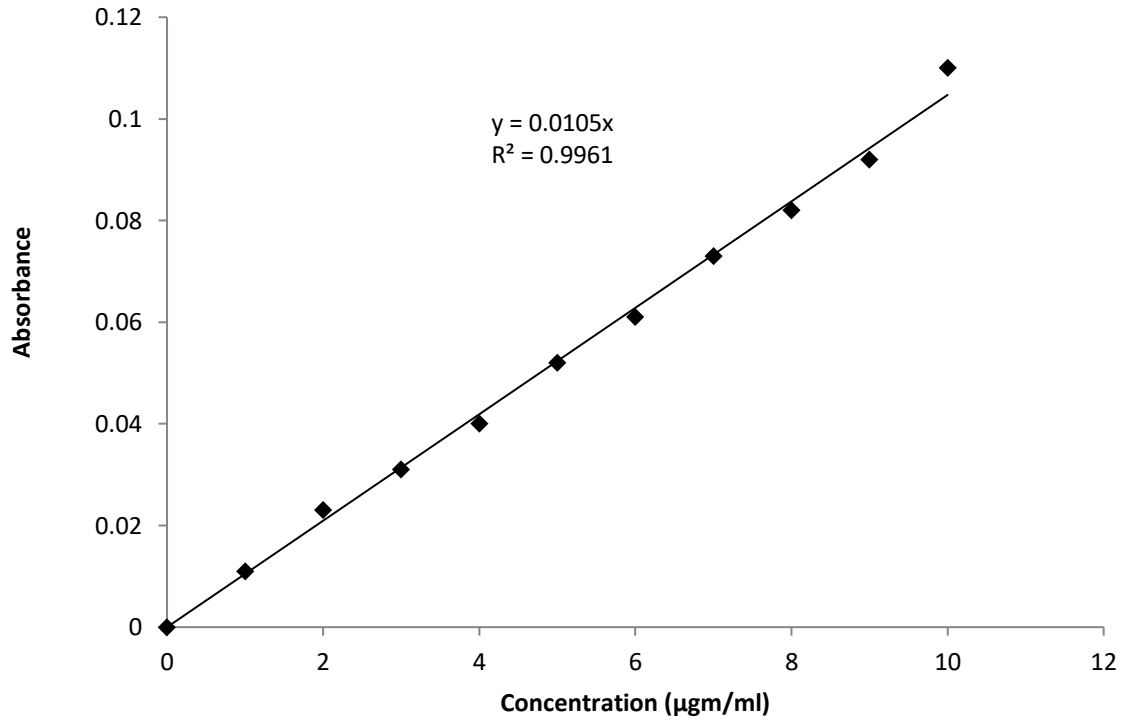


Figure 2: Standard curve of Levofloxacin hemihydrate in 0.1 N HCl

PERCENTAGE YIELD

Table 5: Percentage yield of Levofloxacin hemihydrate loaded mucoadhesive beads

S.No	Formulation code	Percentage yield (Mean of three values ± SD)
1	LM1	33.18±0. 91
2	LM 2	47.54±0.37
3	LM 3	60.15±0.98
4	LM 5	56.73±0.86
5	LM 5	71.32±0.79
6	LM 6	81.81±0.71
7	LM 7	62.25±0.96
8	LM 8	78.34±0.87
9	LM 9	88.16±0.82

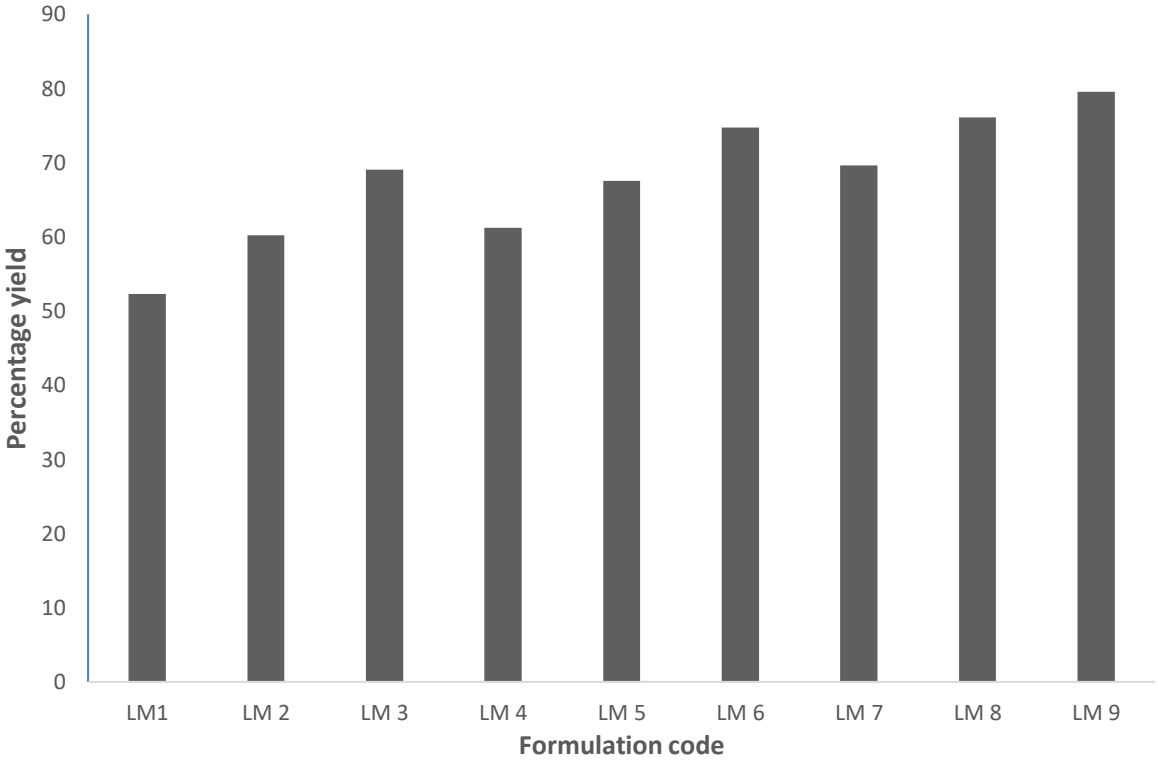


Figure 3: Percentage yield of Levofloxacin hemihydrate loaded mucoadhesive beads

DRUG CONTENT

Table 6: Drug content of levofloxacin hemihydrate loaded mucoadhesive beads

S.No	Formulation code	Theoretical drug content (%)	Practical drug content (%) (Mean of three values ± SD)
1	LM1	55.60	20.58±0.82
2	LM 2	45.45	21.56±0.33
3	LM 3	38.46	22.64±0.42
4	LM 5	55.60	24.56±0.41
5	LM 5	45.45	25.85±0.34
6	LM 6	38.46	26.45±0.65
7	LM 7	55.60	27.95±0.44
8	LM 8	45.45	28.79±0.49
9	LM 9	38.46	29.90±0.61

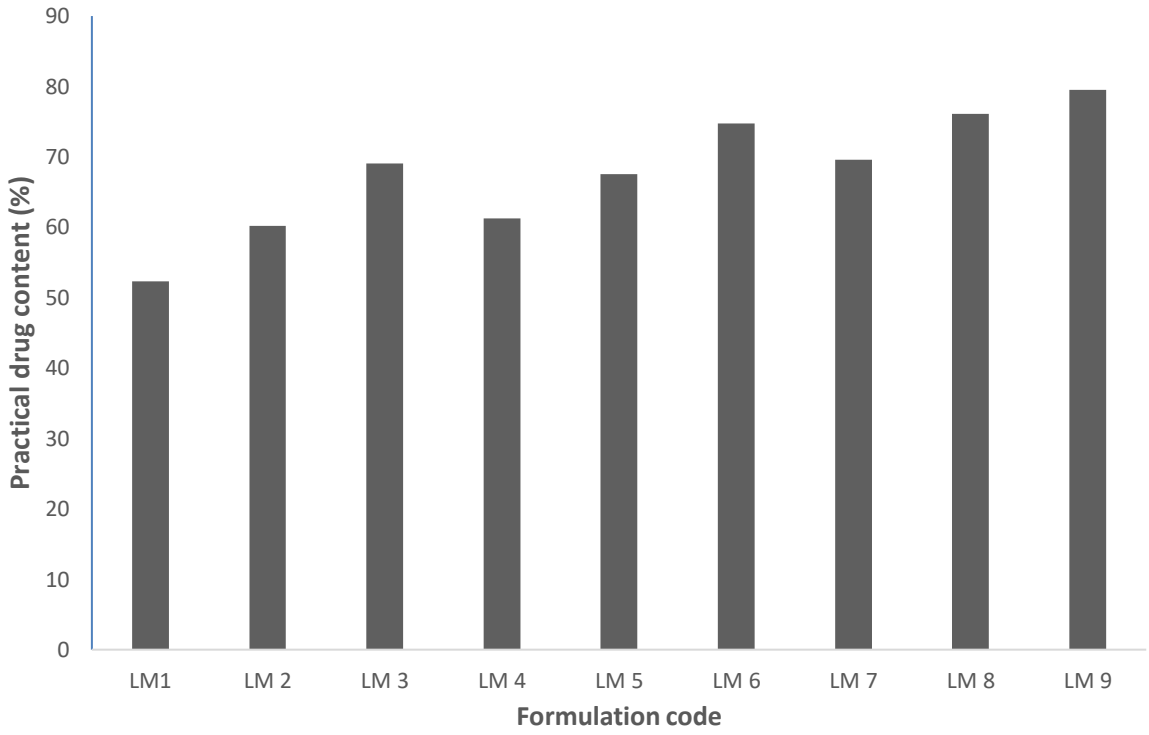


Figure 4: Drug content of levofloxacin hemihydrate loaded mucoadhesive beads

ENCAPSULATION EFFICIENCY

Table 7: Encapsulation efficiency of Levofloxacin hemihydrate loaded mucoadhesive beads

S.No	Formulation code	Percentage drug loaded (Mean of three values ± SD)
1	LM1	52.31±0.23
2	LM 2	60.22±0.81
3	LM 3	69.04±0.54
4	LM 5	61.24±0.92
5	LM 5	67.51±0.73
6	LM 6	74.74±0.85
7	LM 7	69.62±0.71
8	LM 8	76.11±0.24
9	LM 9	79.55±0.55

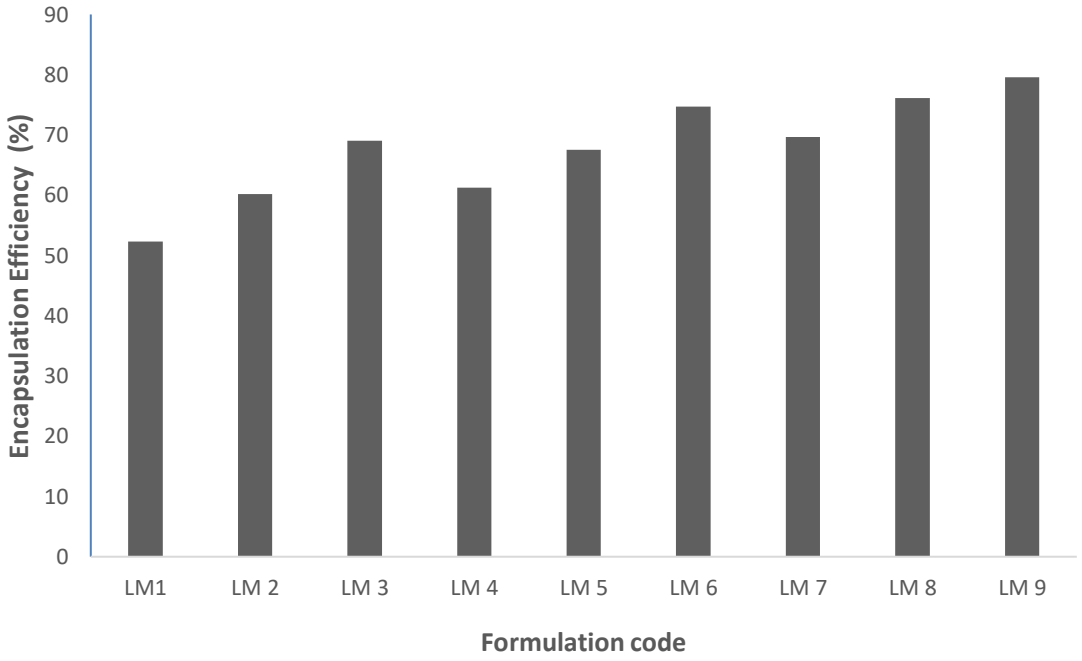


Figure 5: Encapsulation efficiency of Levofloxacin hemihydrate loaded mucoadhesive beads

MUCOADSIVENESS STUDY

Table 8: Mucoadsivenes of Levofloxacin hemihydrate loaded mucoadhesive beads

S.No	Formulation code	Mucin Adsorption (%) (Mean of three values ± SD)
1	LM1	46.52±0.57
2	LM 2	56.78±0.23
3	LM 3	66.25±0.79
4	LM 5	56.21±0.67
5	LM 5	69.55±0.55
6	LM 6	80.47± 0.33
7	LM 7	63.84±0.25
8	LM 8	75.64±0.29
9	LM 9	84.51±0.74

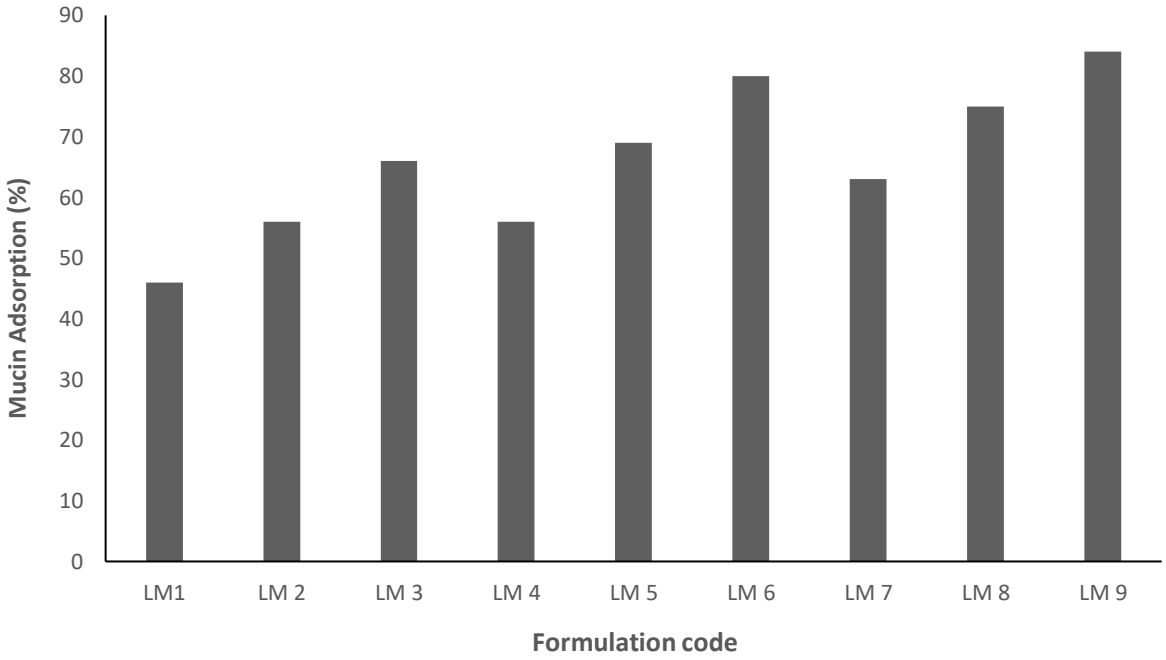


Figure 6: Mucoadsivenes of Levofloxacin hemihydrate loaded mucoadhesive bead

PARTICLE SIZE DISTRIBUTION

Table 9: Particle size distribution of Levofloxacin hemihydrate loaded mucoadhesive beads

S.No	Formulation code	Particle size (µm) (Mean of three values ± SD)
1	LM1	141.2±0.78
2	LM 2	223.67±0.73
3	LM 3	291.62±0.55
4	LM 5	235.54±0.45
5	LM 5	319.54±0.76
6	LM 6	402.47±0.98
7	LM 7	289.78±0.24
8	LM 8	362.21±0.76
9	LM 9	451.04±0.90

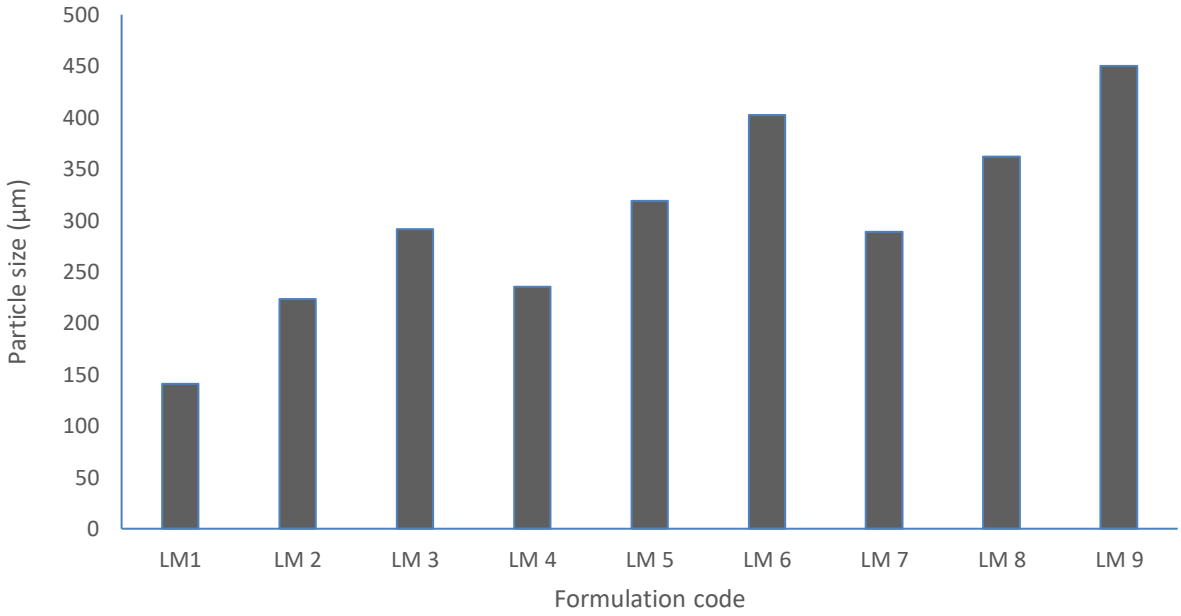


Figure 7: Particle size distribution of Levofloxacin hemihydrate loaded mucoadhesive bead

COMPATIBILITY STUDIES

FOURIER TRANSFORM INFRARED SPECTROPHOTOMETRY (FTIR) STUDIES

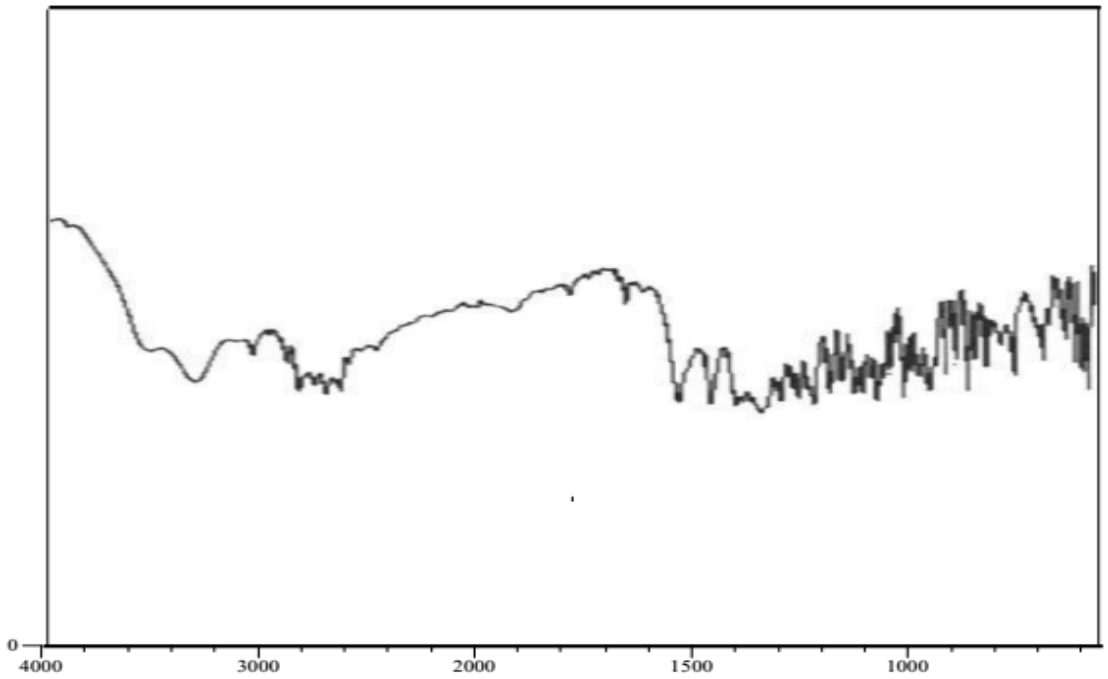


Figure 10: FTIR spectra of Levofloxacin hemihydrate

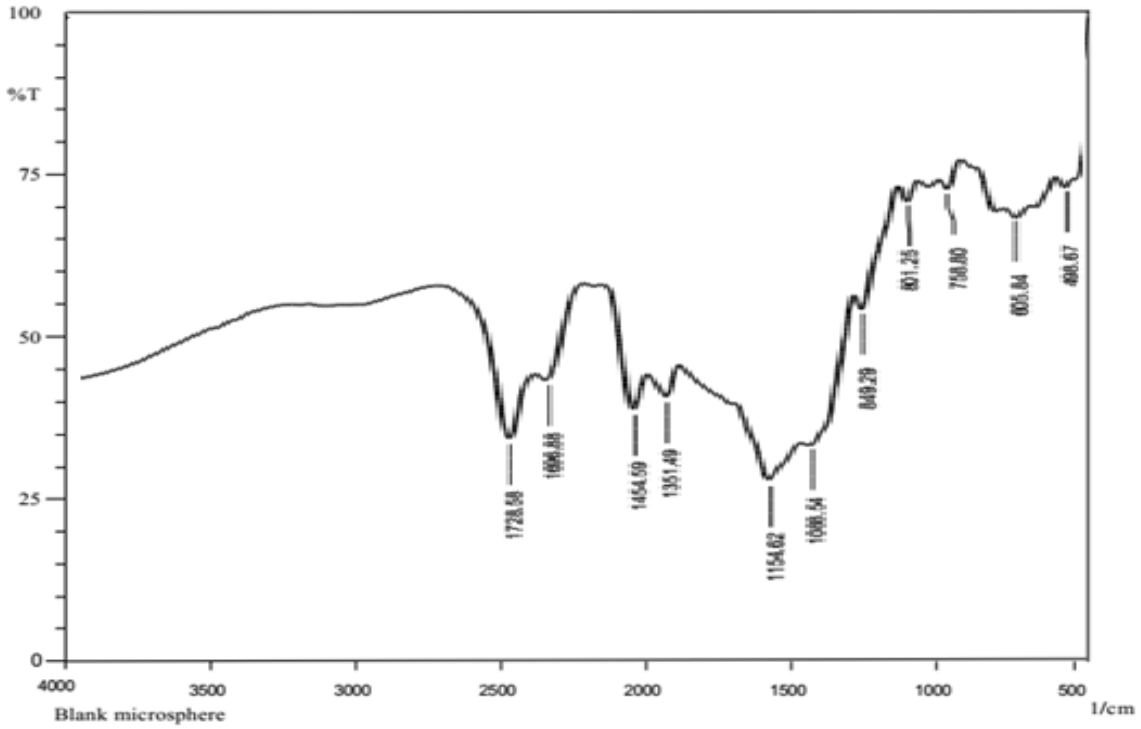


Figure 11: FTIR spectra of Blank mucoadhesive beads

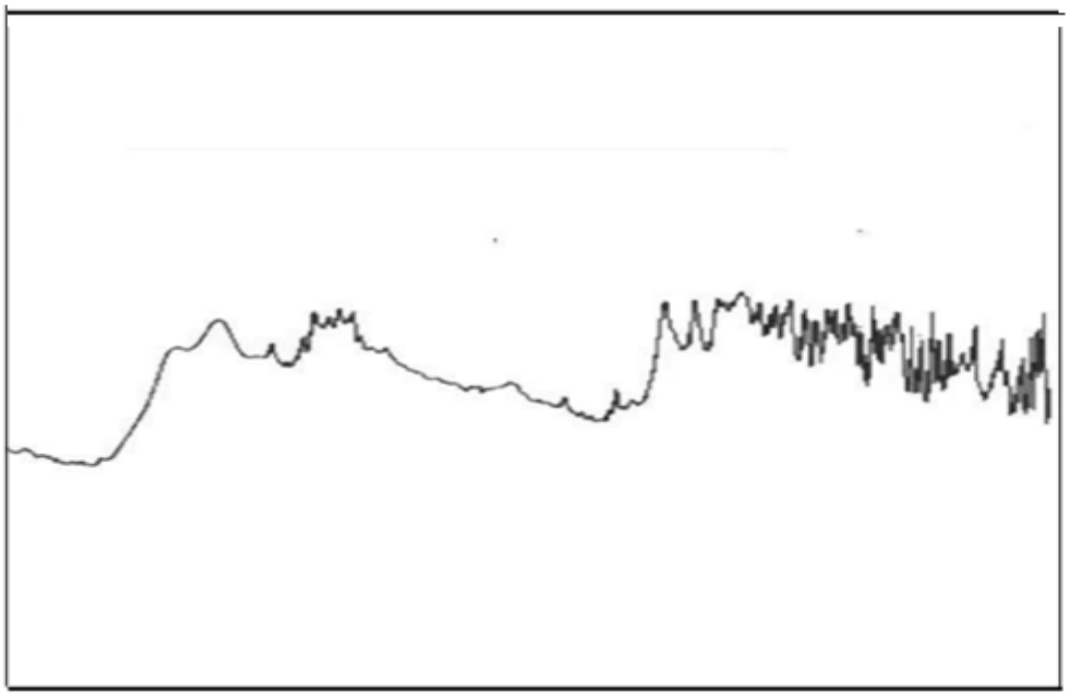


Figure 12 FTIR spectra of Levofloxacin hemihydrate loaded mucoadhesive beads[LM5]

Table 10: Characteristic IR bands of Levofloxacin hemihydrate in mucoadhesive beads.

Principle peaks	**Levofloxacin hemihydrate (cm^{-1})**	**Levofloxacin hemihydrate loaded beads**
–COOH monomeric stretching and bonding	3269 and 1045 cm^{-1}	All the above peaks are present in drug-loaded formulations that confirm the presence of drug in the polymer without any interaction.
alkanes $–CH_3$ and aromatic rings	2846 and 1618 cm^{-1}	
C=O stretching vibration of the COOH group	1721 cm^{-1}	
C– F	835 cm^{-1}	

DIFFERENTIAL SCANNING CALORIMETERY (DSC) STUDIES

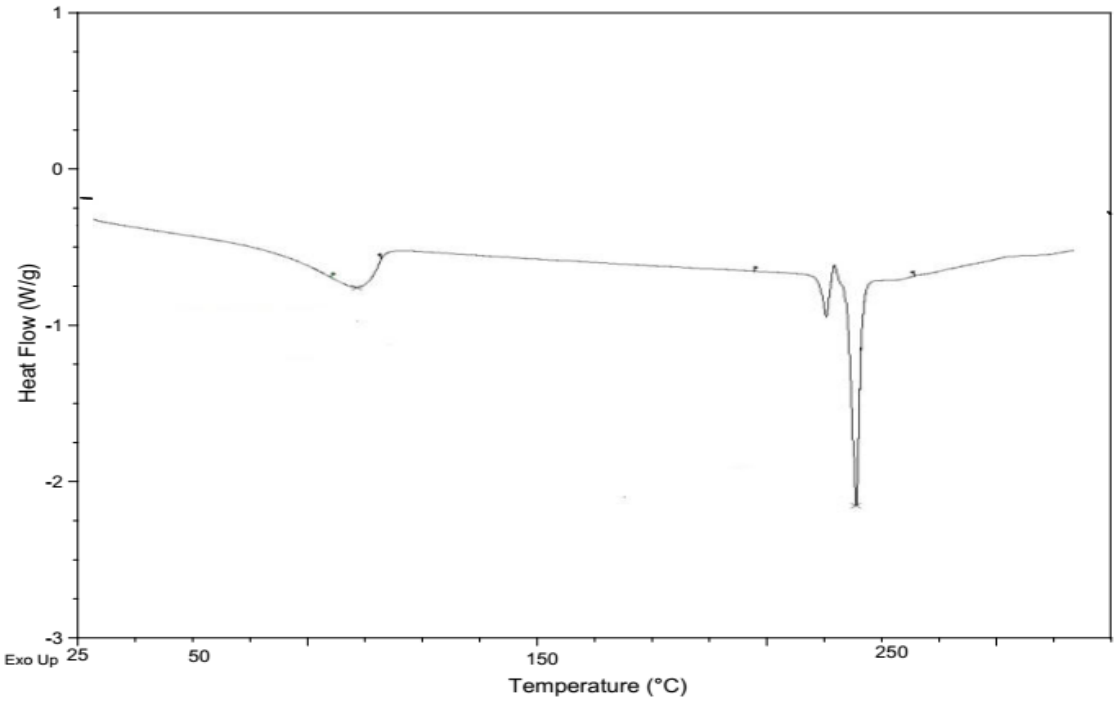


Figure 13: DSC spectra of Levofloxacin hemihydrate

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

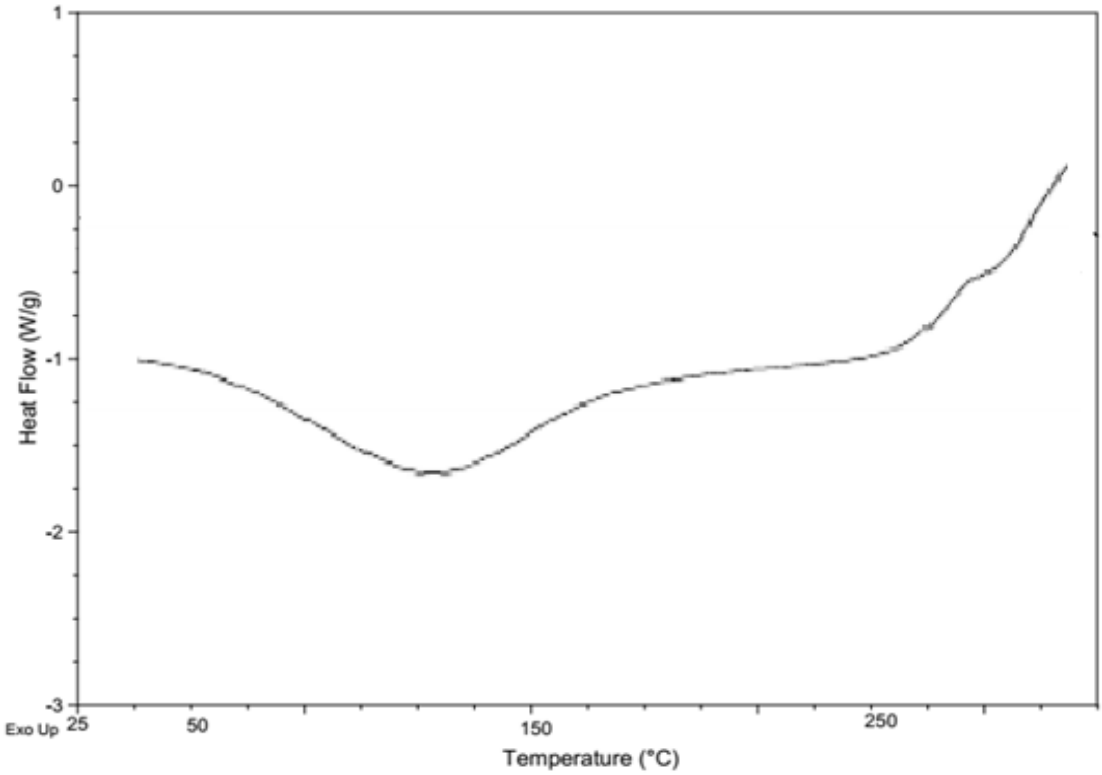


Figure 14: DSC spectra of Dsc of Sodium alginate

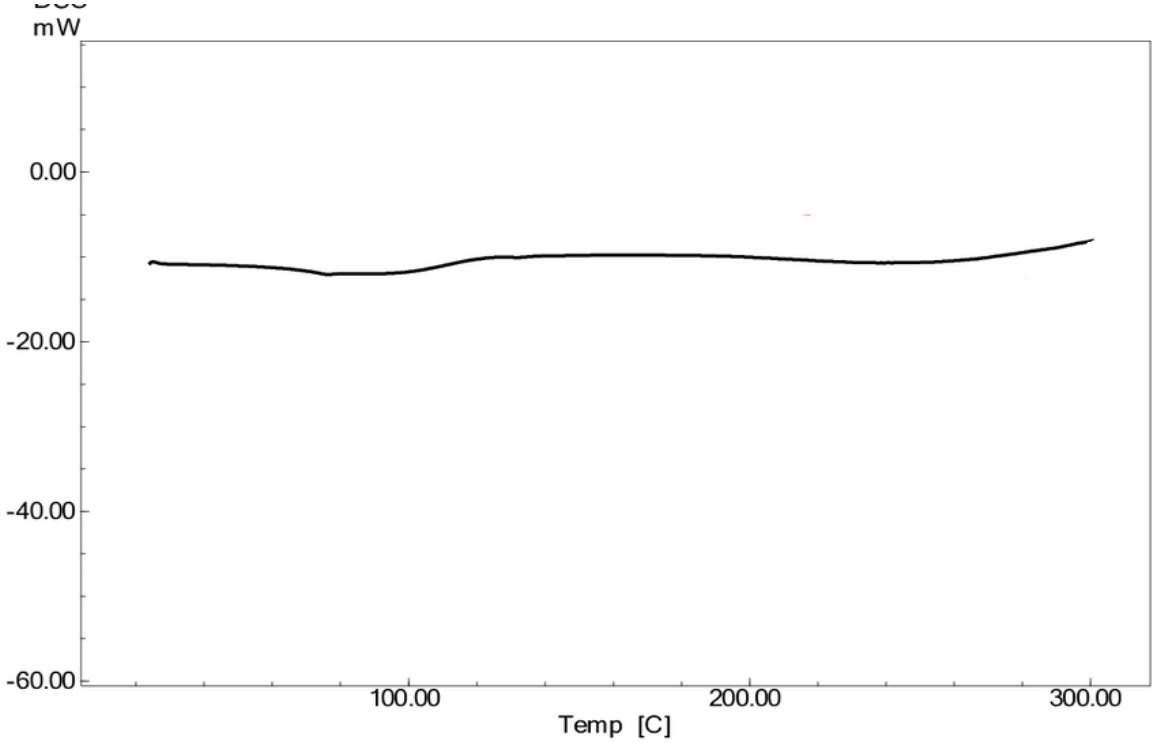


Figure 15: DSC spectra of Dsc of Carbopol 974P

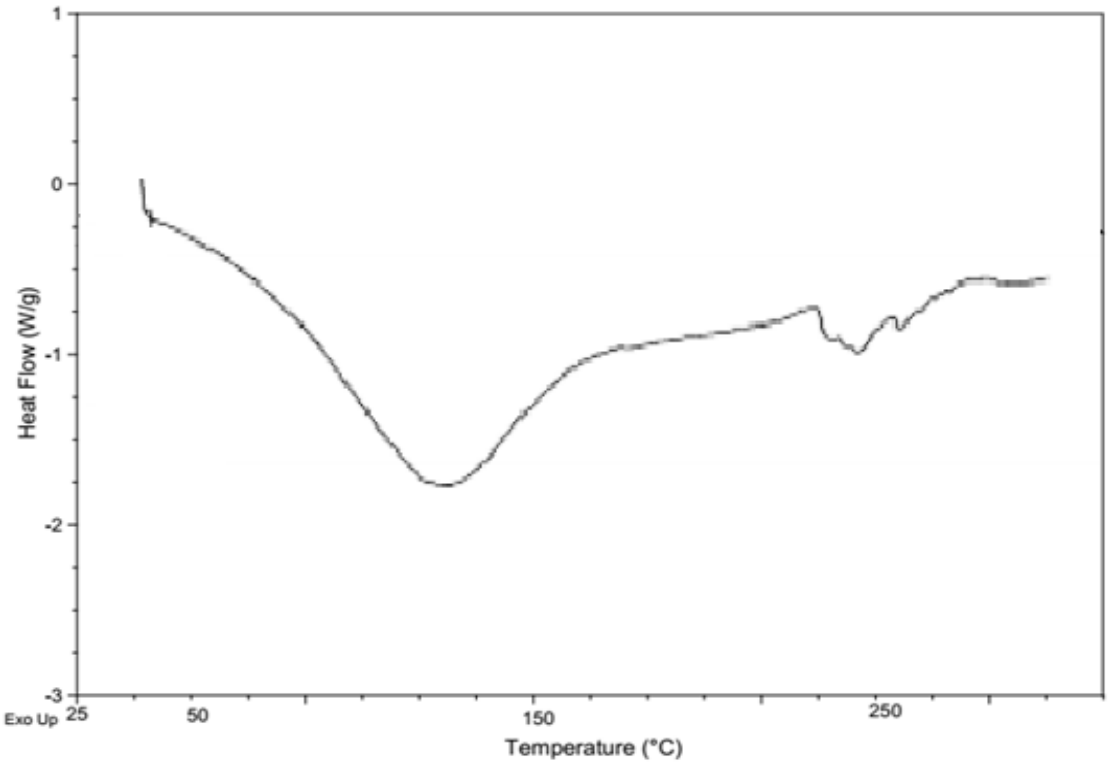


Figure 16: DSC spectra of blank beads

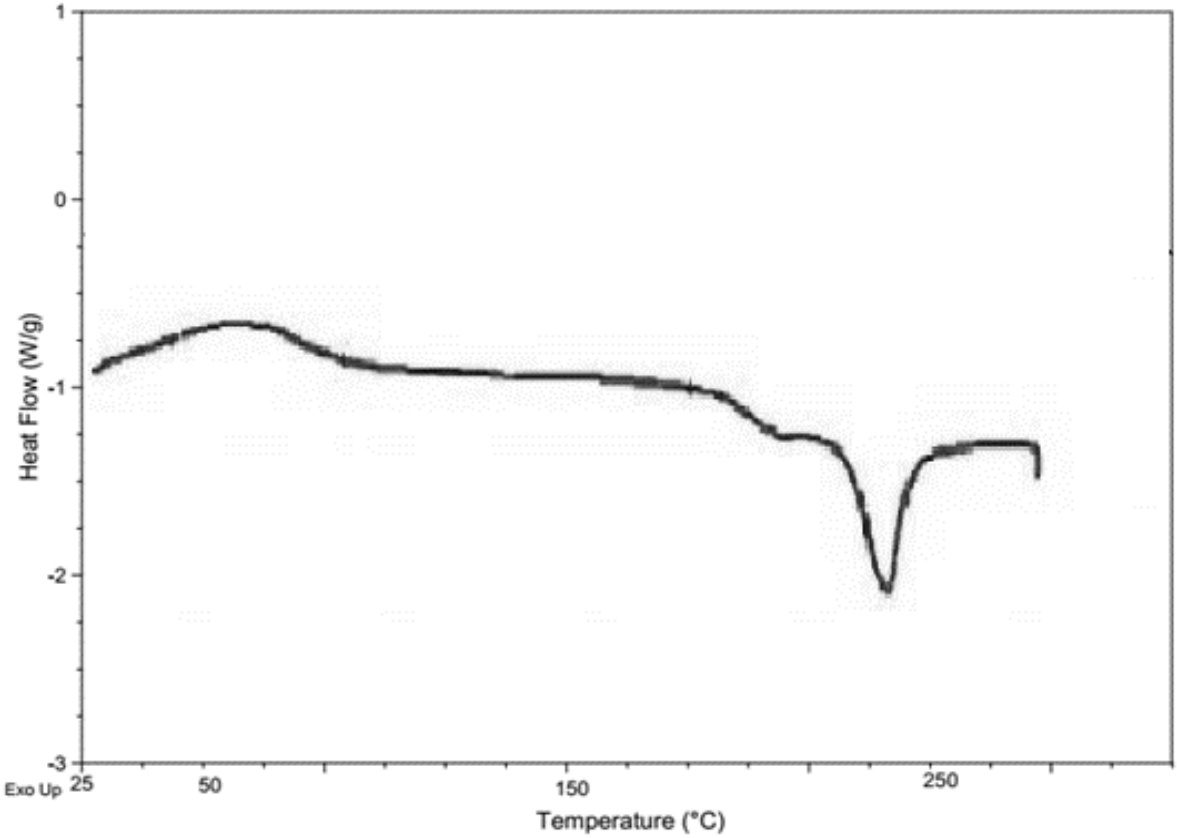


Figure 17: DSC spectra of Levofloxacin hemihydrate loaded mucoadhesive beads[LM5].

SCANNING ELECTRON MICROGRAPH OF LEVOFLOXACIN HEMIHYDRATE LOADED MUCOADHESIVE BEADS

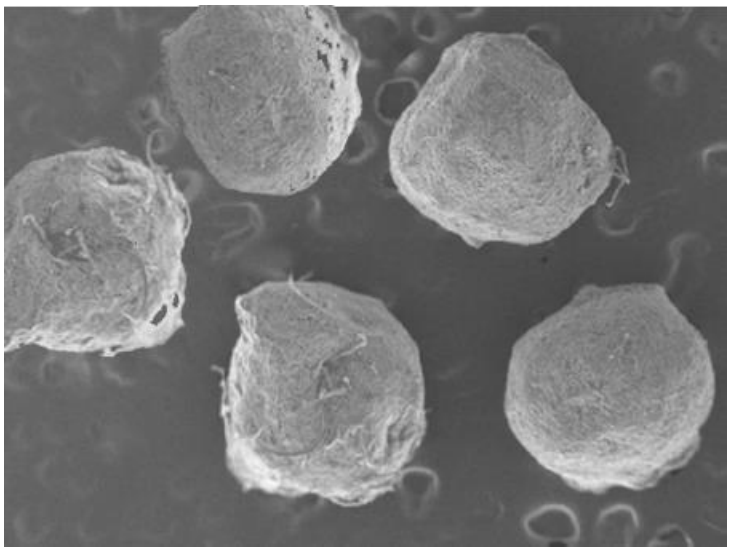


Figure 8: SEM photograph of formulation LM 5

Figure 9: SEM photograph of formulation LM 5(Surface View)

IN VITRO DISSOLUTION STUDIES

Table 11: *In vitro* release profile of Levofloxacin hemihydrate loaded mucoadhesive beads (Formulation LM1)

Time (hours)	Cumulative Percentage drug Released*	Standard Deviation**
0	0	0
1	52.08	1.14
2	80.33	1.03
3	99.62	0.15
4	-	-
5	-	-
6	-	-
7	-	-

*Each reading is an average of three determinations

**Standard deviation of three determinations

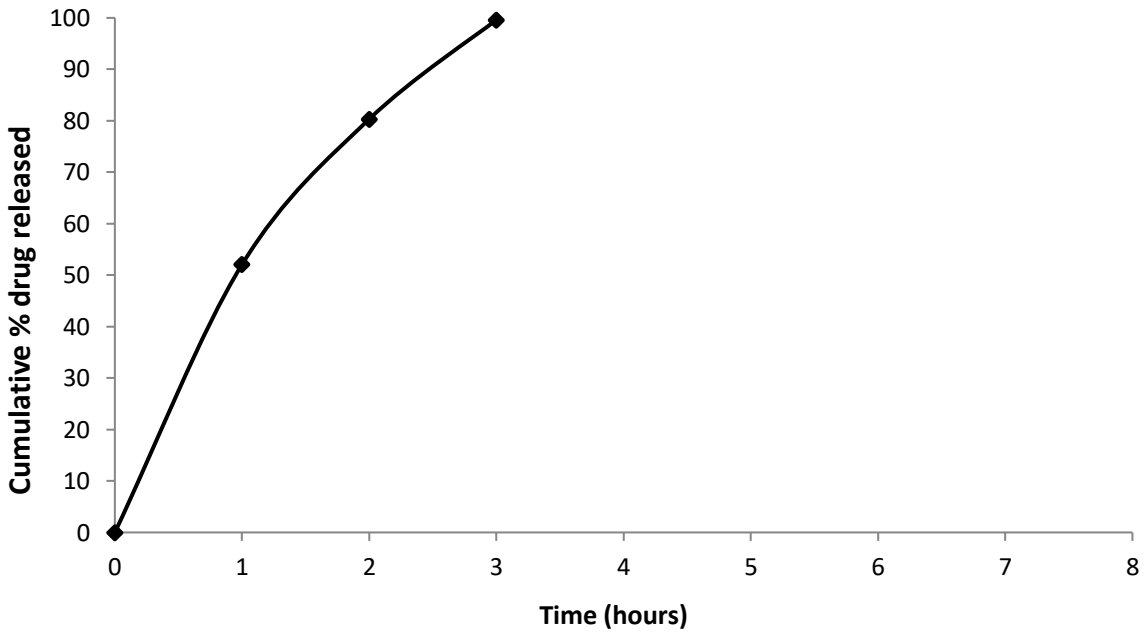


Figure 18: *In vitro* release profile of Levofloxacin hemihydrate loaded mucoadhesive beads (Formulation LM1) (Bars represent mean of three values ± SD)

Table 12: *In vitro* release profile of Levofloxacin hemihydrate loaded mucoadhesive beads (Formulation LM2)

Time (hours)	Cumulative Percentage drug Released*	Standard Deviation**
0	0	0
1	44	1.65
2	70	1.32
3	92	1.37
4	99.69	0.11
5	-	-
6	-	-
7	-	-

*Each reading is an average of three determinations

**Standard deviation of three determinations

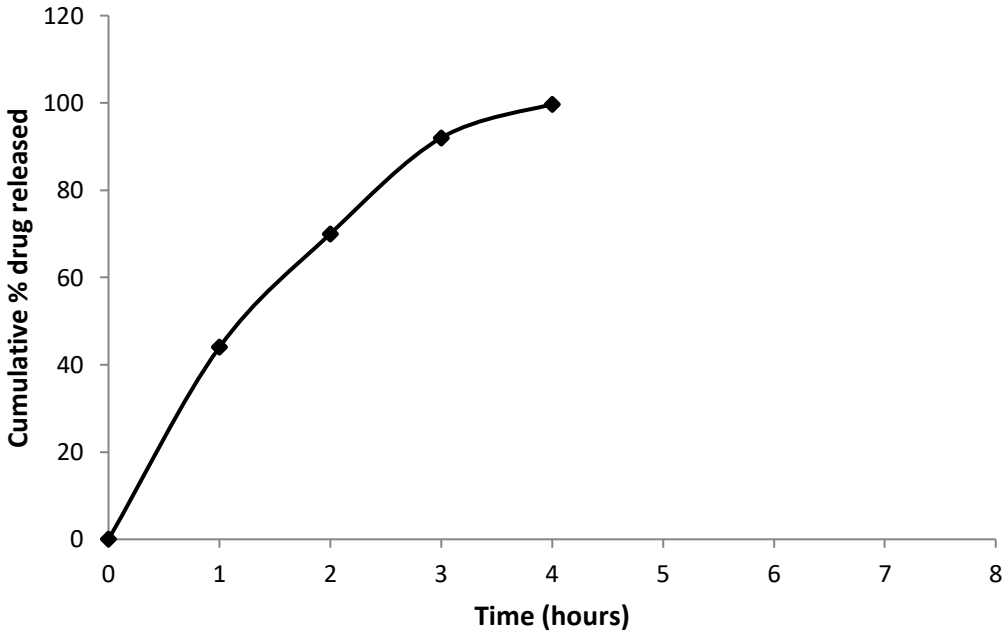


Figure 19: *In vitro* release profile of Levofloxacin hemihydrate loaded mucoadhesive beads (Formulation LM2) (Bars represent mean of three values ± SD)

Table 13: *In vitro* release profile of Levofloxacin hemihydrate loaded mucoadhesive beads (Formulation LM3)

Time (hours)	Cumulative Percentage drug Released*	Standard Deviation**
0	0	0
1	39	2.41
2	62	2.51
3	83.41	1.24
4	93	1.74
5	99.78	0.11
6	-	-
7	-	-

*Each reading is an average of three determinations

**Standard deviation of three determinations

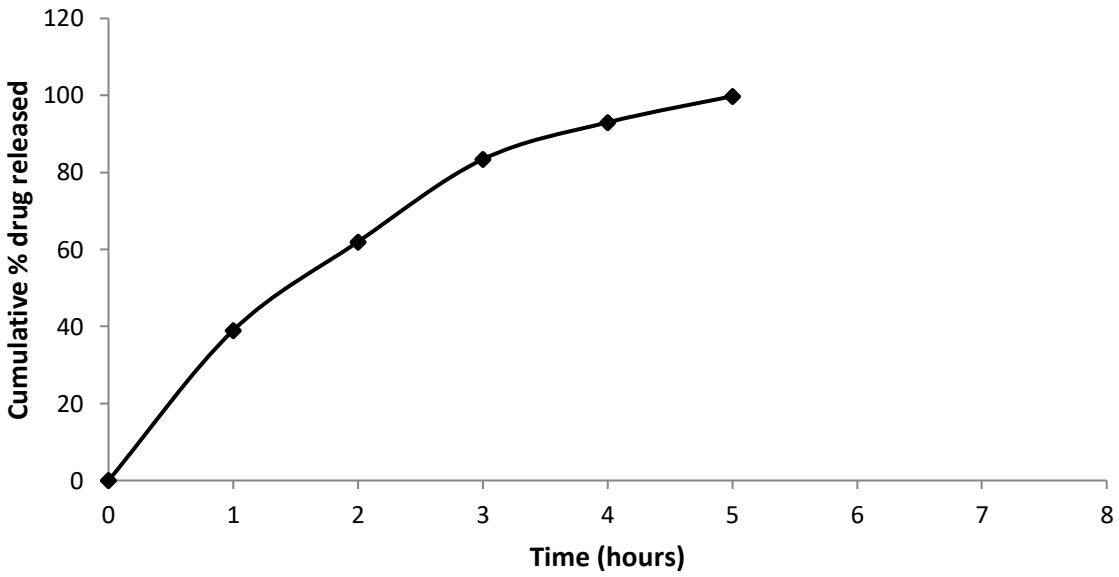


Figure 20: *In vitro* release profile of Levofloxacin hemihydrate loaded mucoadhesive beads (Formulation LM3) (Bars represent mean of three values ± SD)

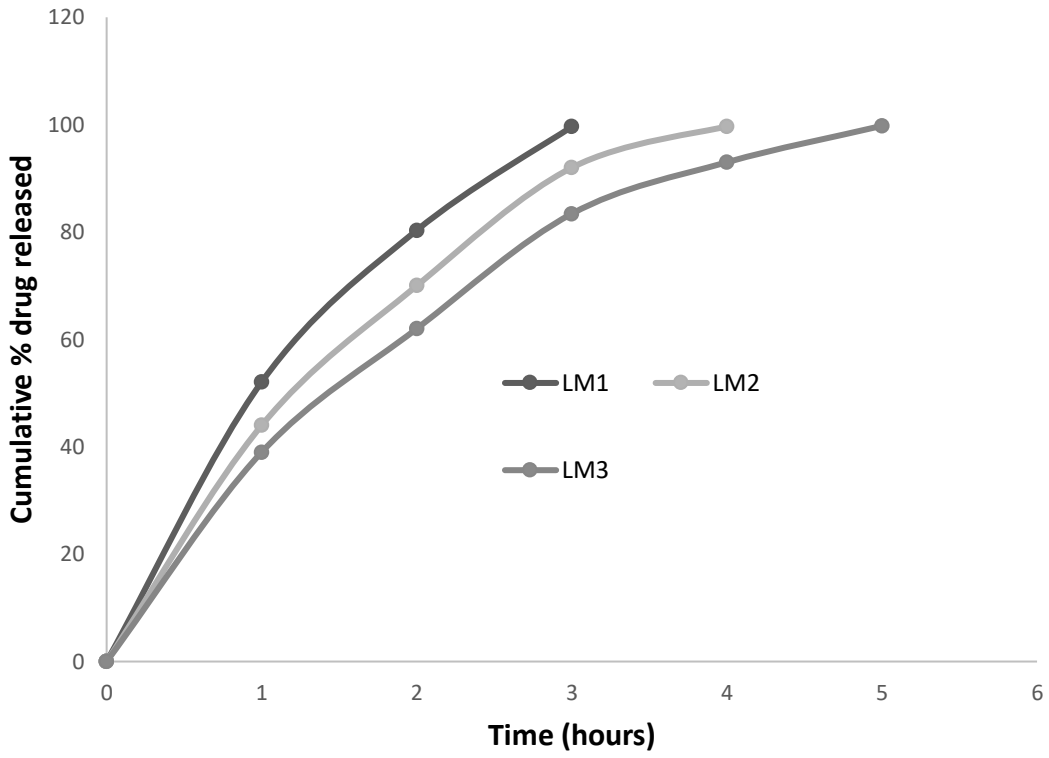


Figure 21: Effect of Different concentration of sodium alginate on drug release (2 % w/v)

Table 14: *In vitro* release profile of am Levofloxacin hemihydrate oxicillin trihydrate loaded mucoadhesive beads (Formulation LM4)

Time (hours)	Cumulative Percentage drug Released*	Standard Deviation**
0	0	0
1	45.61	1.23
2	64.07	1.41
3	81.29	1.51
4	99.25	2.11
5		
6		
7		

*Each reading is an average of three determinations

**Standard deviation of three determination

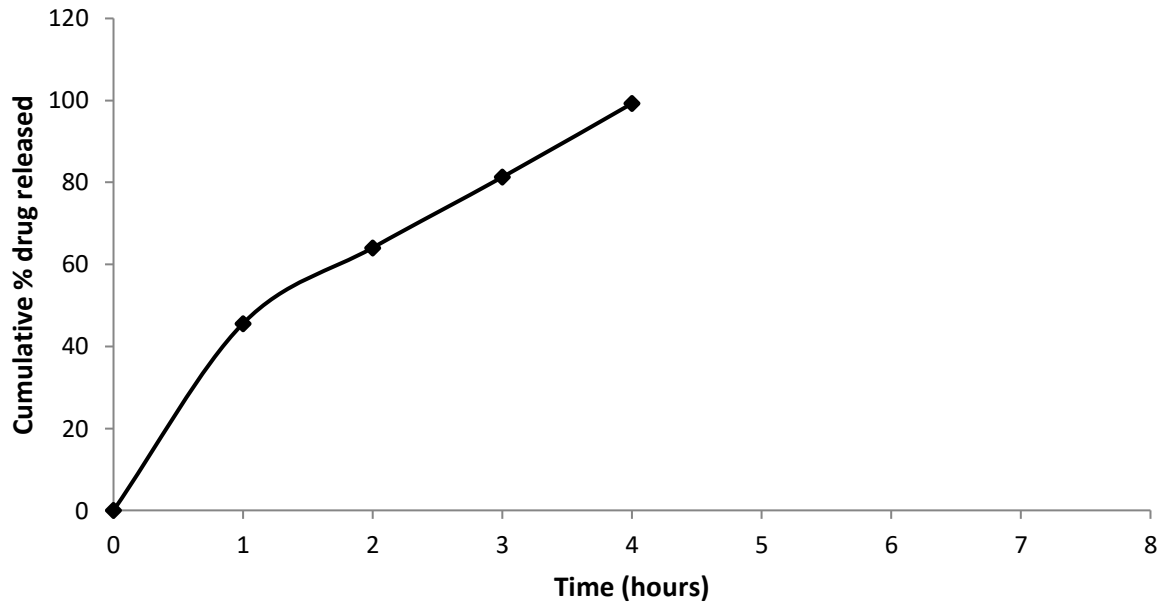


Figure 22: *In vitro* release profile of Levofloxacin hemihydrate loaded mucoadhesive beads (Formulation LM4) (Bars represent mean of three values ± SD)

Table 15: *In vitro* release profile of Levofloxacin hemihydrate loaded mucoadhesive beads (Formulation LM5)

Time (hours)	Cumulative Percentage drug Released*	Standard Deviation**
0	0	0
1	37	1.24
2	54	1.57
3	63.67	2.31
4	71.81	0.18
5	84.22	1.23
6	99.62	0.51
7		-

*Each reading is an average of three determinations

**Standard deviation of three determinations

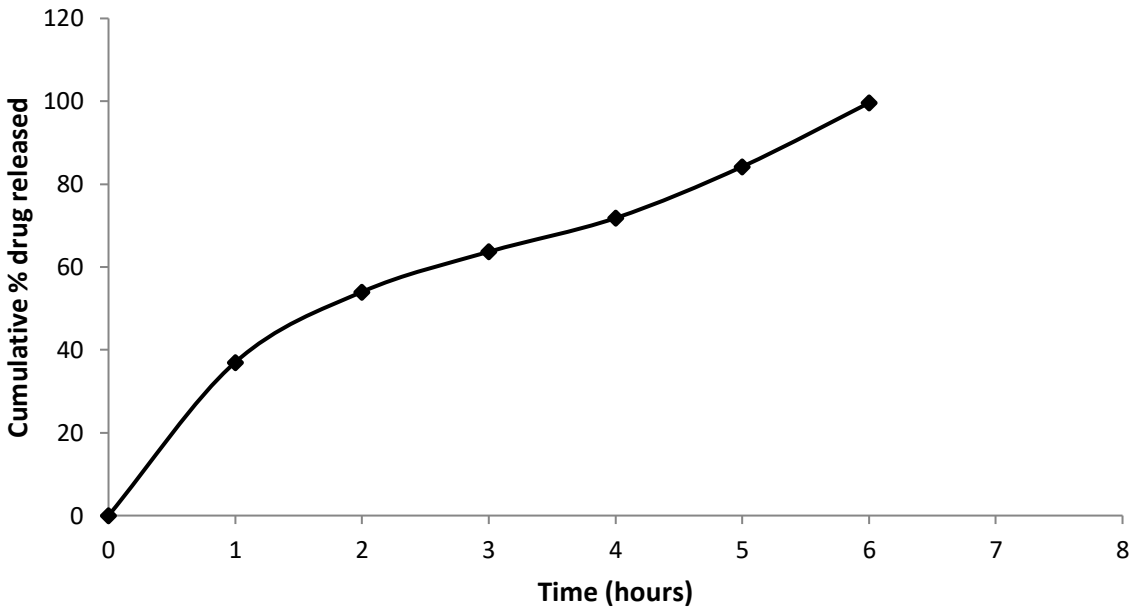


Figure 23: *In vitro* release profile of Levofloxacin hemihydrate loaded mucoadhesive beads (Formulation LM5) (Bars represent mean of three values ± SD)

Table 16: *In vitro* release profile of Levofloxacin hemihydrate loaded mucoadhesive beads (Formulation LM6)

Time (hours)	Cumulative Percentage drug Released*	Standard Deviation**
0	0	0
1	31.92	1.31
2	49.17	1.62
3	59.41	1.49
4	67.17	2.67
5	78.75	2.45
6	85.77	1.11
7	94.46	0.51

*Each reading is an average of three determinations

**Standard deviation of three determinations

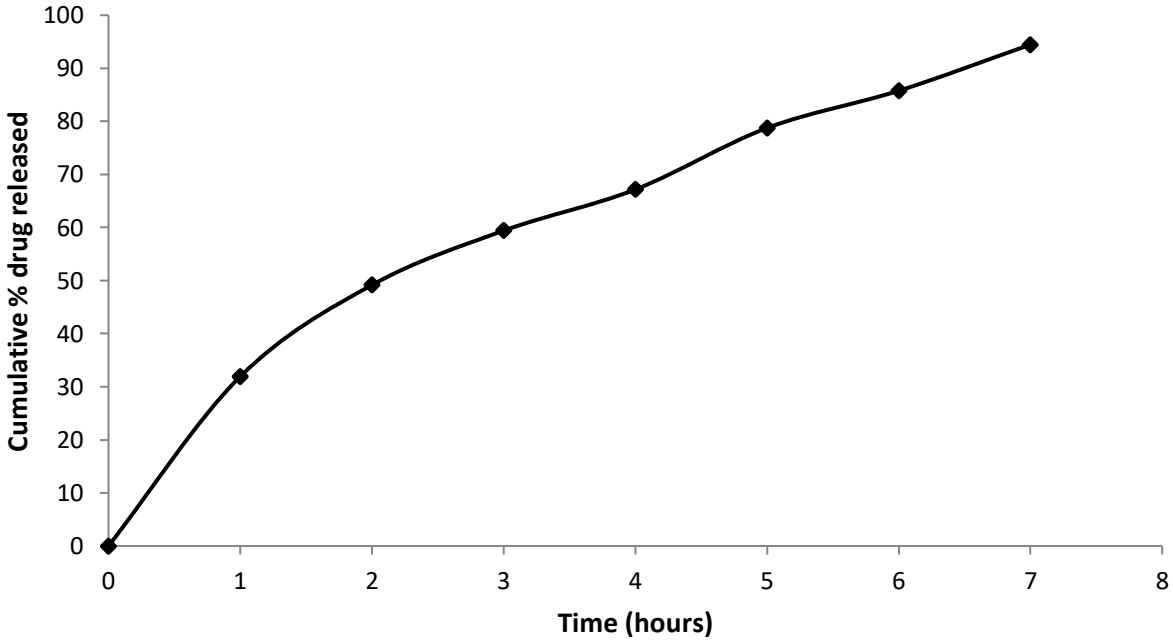


Figure 24: *In vitro* release profile of Levofloxacin hemihydrate loaded mucoadhesive beads (Formulation LM6) (Bars represent mean of three values ± SD)

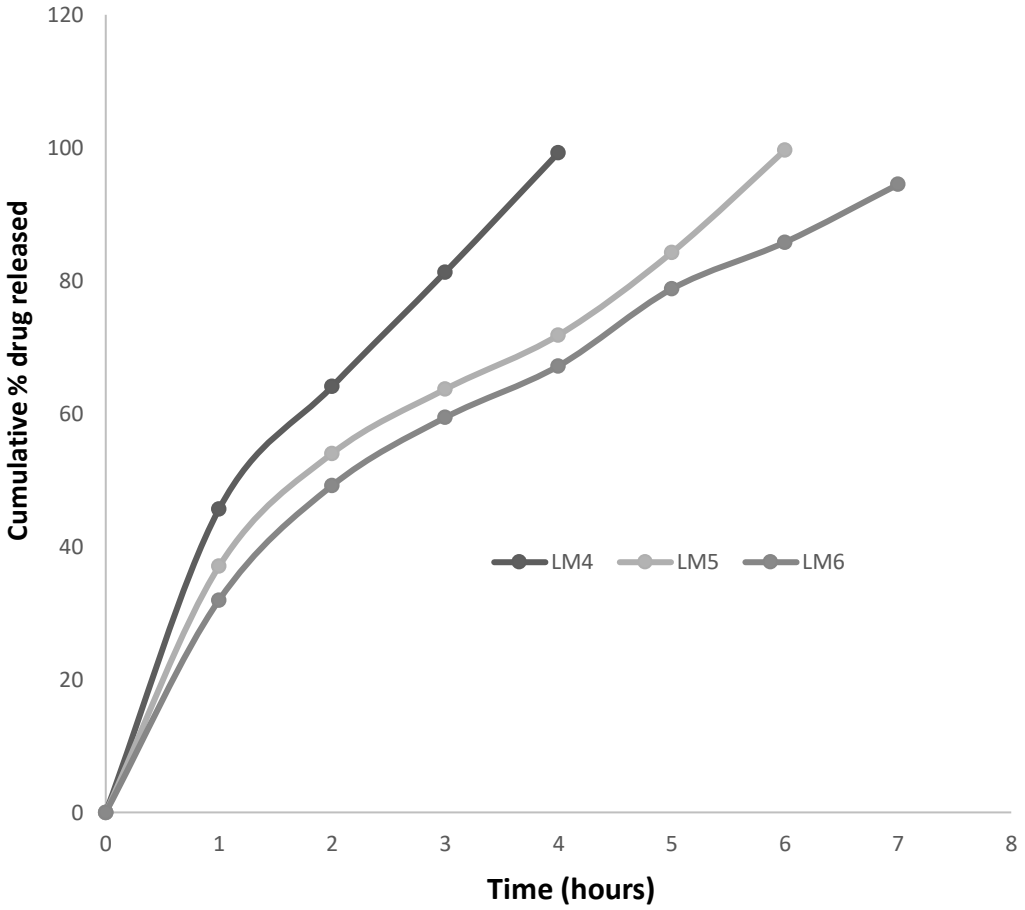


Figure 25: Effect of Different concentration of sodium alginate on drug release (3 % w/v)

Table 17: *In vitro* release profile of Levofloxacin hemihydrate loaded mucoadhesive beads (Formulation LM7)

Time (hours)	Cumulative Percentage drug Released*	Standard Deviation**
0	0	0
1	32.15	1.23
2	52.16	1.34
3	66.67	2.51
4	82.03	2.62
5	99.73	0.22
6		
7		

*Each reading is an average of three determinations

**Standard deviation of three determinations

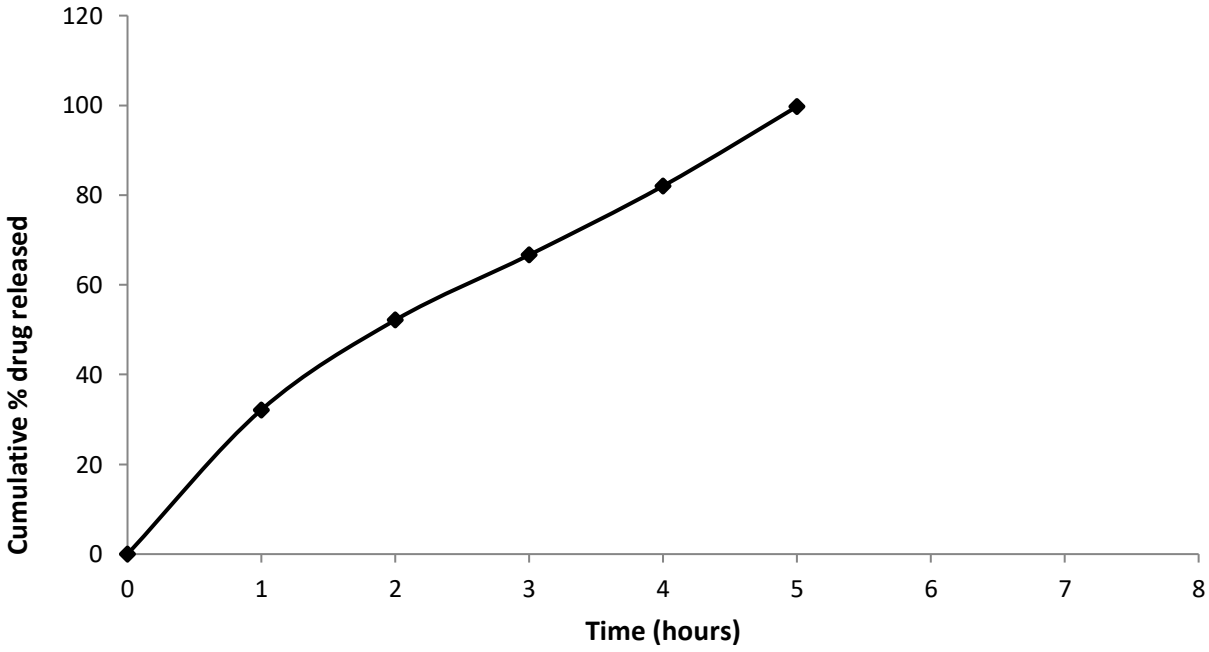


Figure 26: *In vitro* release profile of Levofloxacin hemihydrate loaded mucoadhesive beads (Formulation LM7) (Bars represent mean of three values ± SD)

Table 18: *In vitro* release profile of Levofloxacin hemihydrate loaded mucoadhesive beads (Formulation LM8)

Time (hours)	Cumulative Percentage drug Released*	Standard Deviation**
0	0	0
1	26.51	0.91
2	43	1.50
3	52.25	1.12
4	63.84	0.27
5	74.08	1.21
6	85.15	1.61
7	99.68	0.16

*Each reading is an average of three determinations

**Standard deviation of three determinations

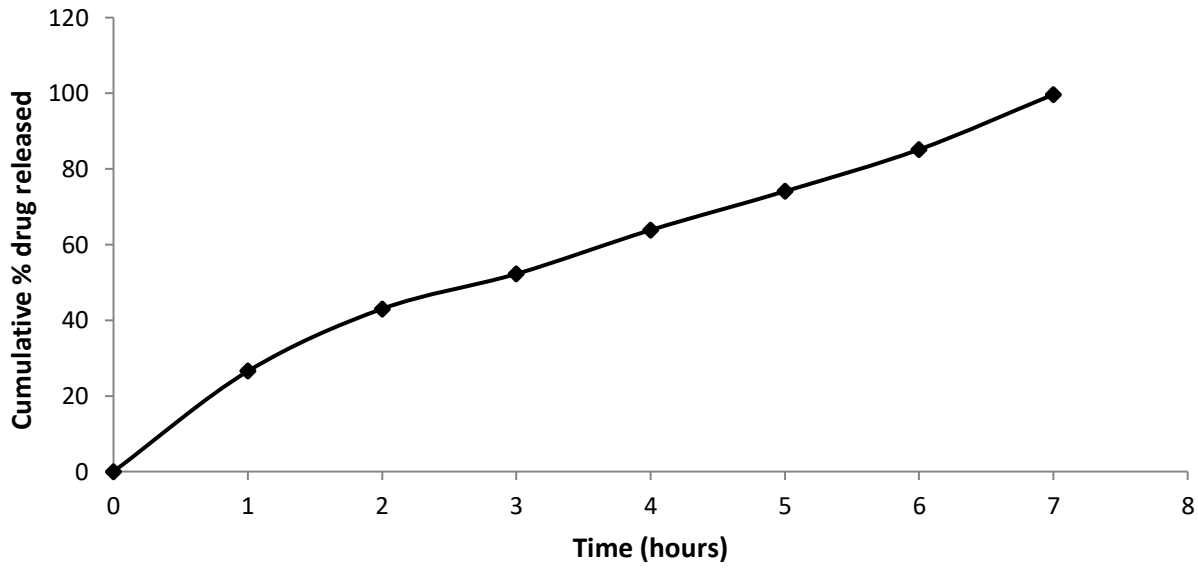


Figure 27: *In vitro* release profile of Levofloxacin hemihydrate loaded mucoadhesive beads (Formulation LM8) (Bars represent mean of three values ± SD)

Table 19: *In vitro* release profile of Levofloxacin hemihydrate loaded mucoadhesive beads [Formulation LM9]

Time (hours)	Cumulative Percentage drug Released*	Standard Deviation**
0	0	0
1	24	0.61
2	39	1.54
3	47	1.17
4	55.76	0.22
5	63.86	1.61
6	71.12	1.23
7	85.81	1.40

*Each reading is an average of three determinations

**Standard deviation of three determinations

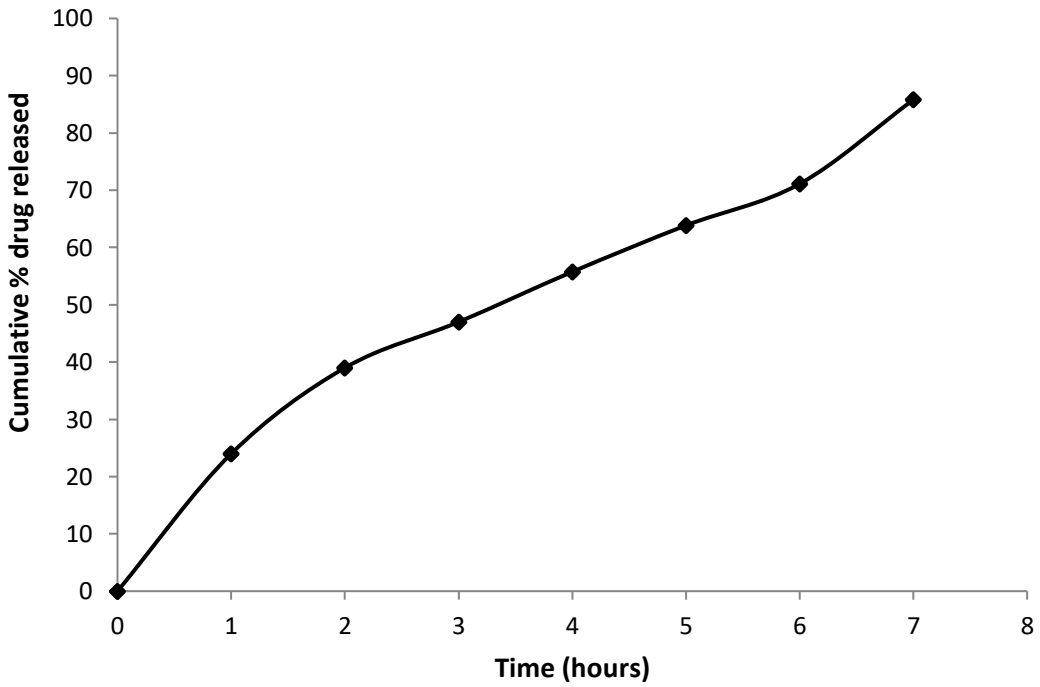


Figure 28: *In vitro* release profile of Levofloxacin hemihydrate loaded mucoadhesive beads [Formulation LM9] [Bars represent mean of three values ± SD]

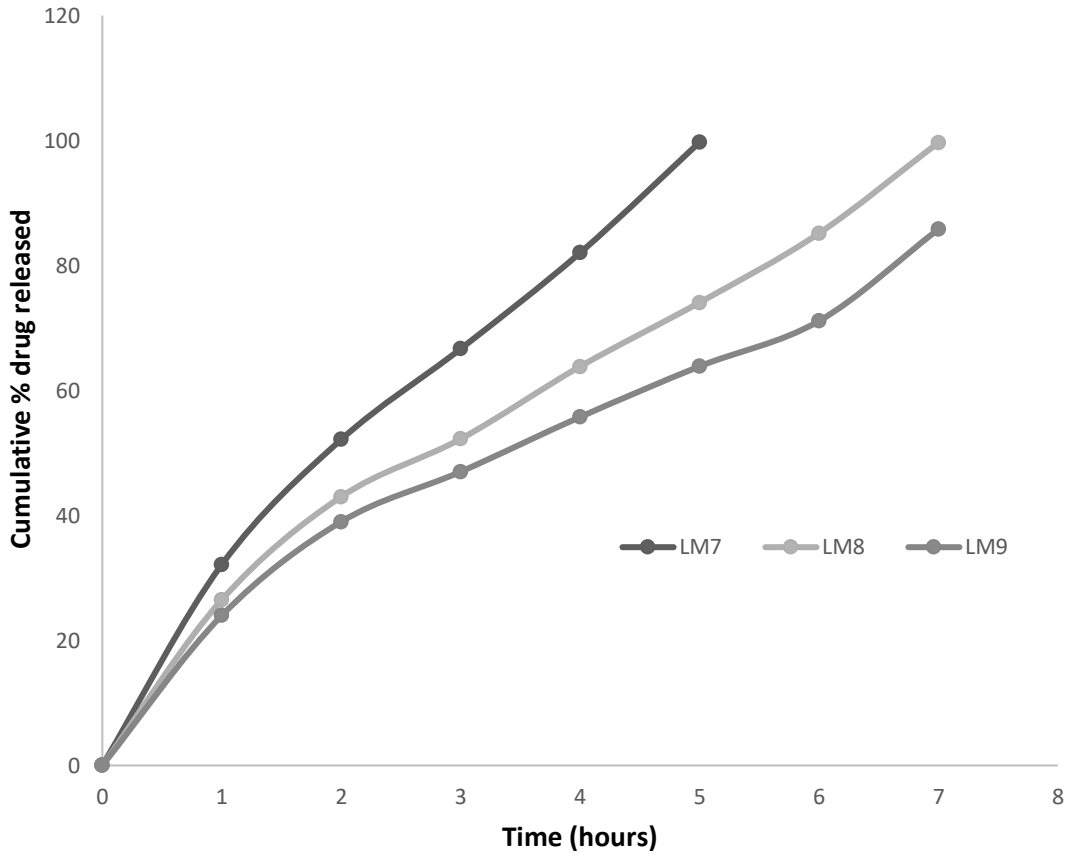


Figure 29: Effect of Different concentration of sodium alginate on drug release (3 % w/v)

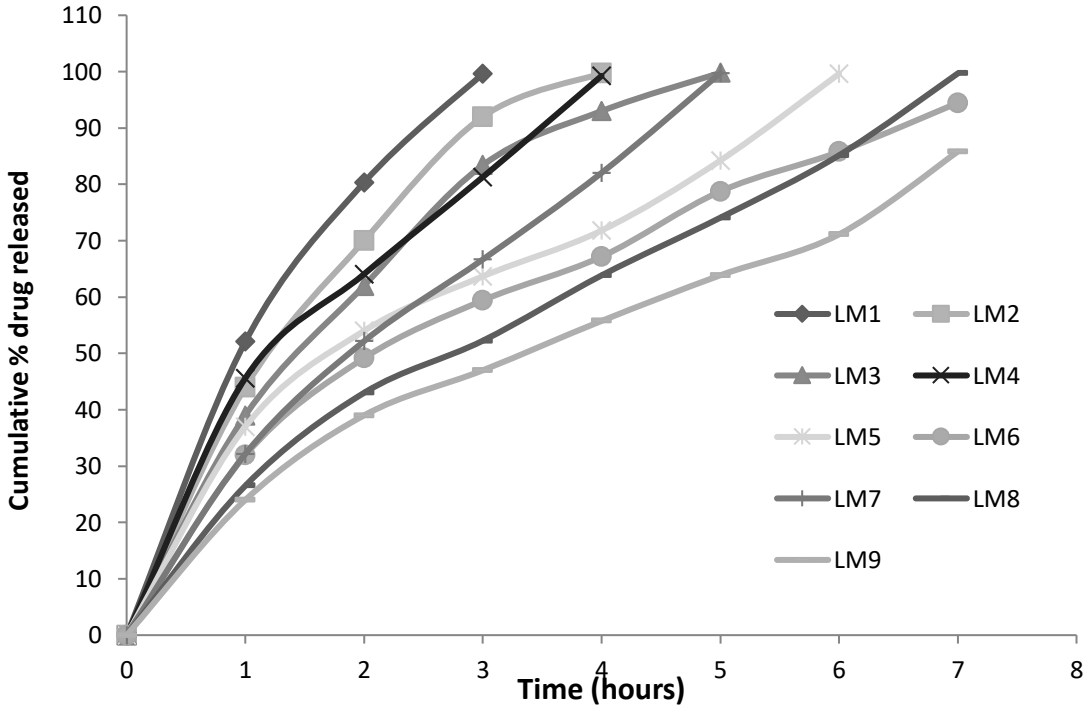


Figure 30: Drug release pattern of various formulatios of levofloxacin Hemihydrate

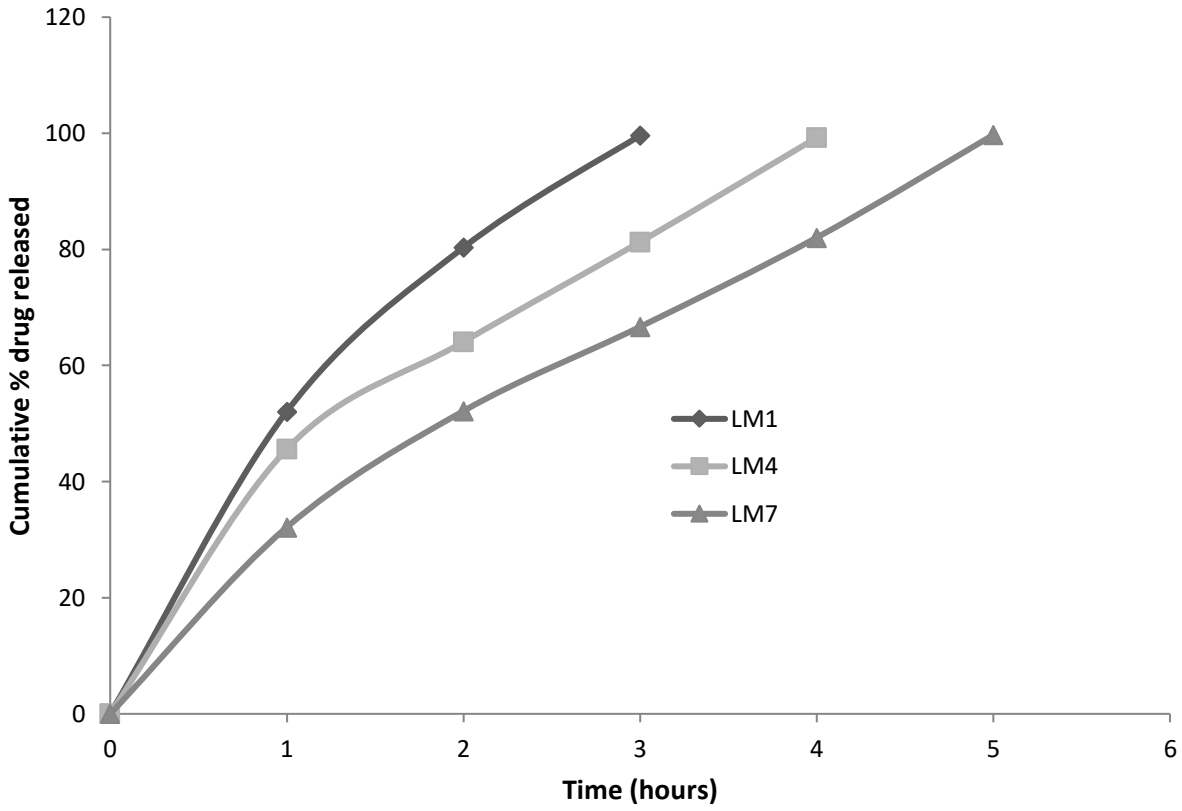


Figure 31: Effect of Different concentration of calcium chloride with 1 %w/w sodium alginate on drug release

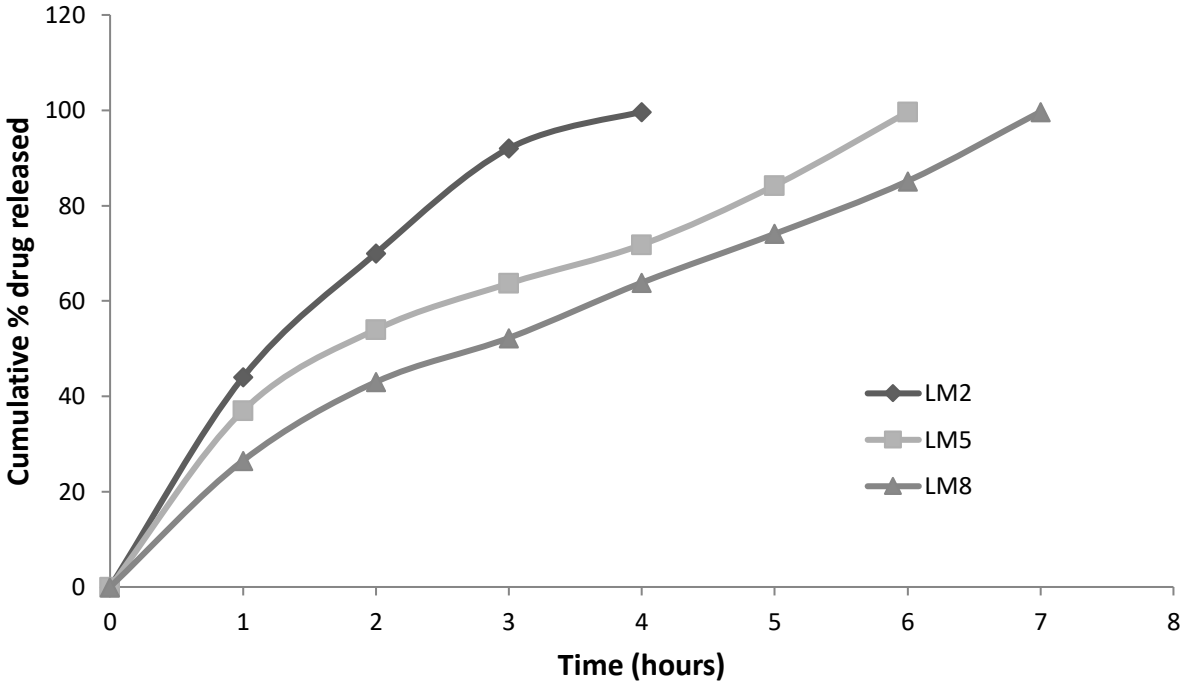


Figure 32: Effect of Different concentration of calcium chloride with 2 %w/w sodium alginate on drug release

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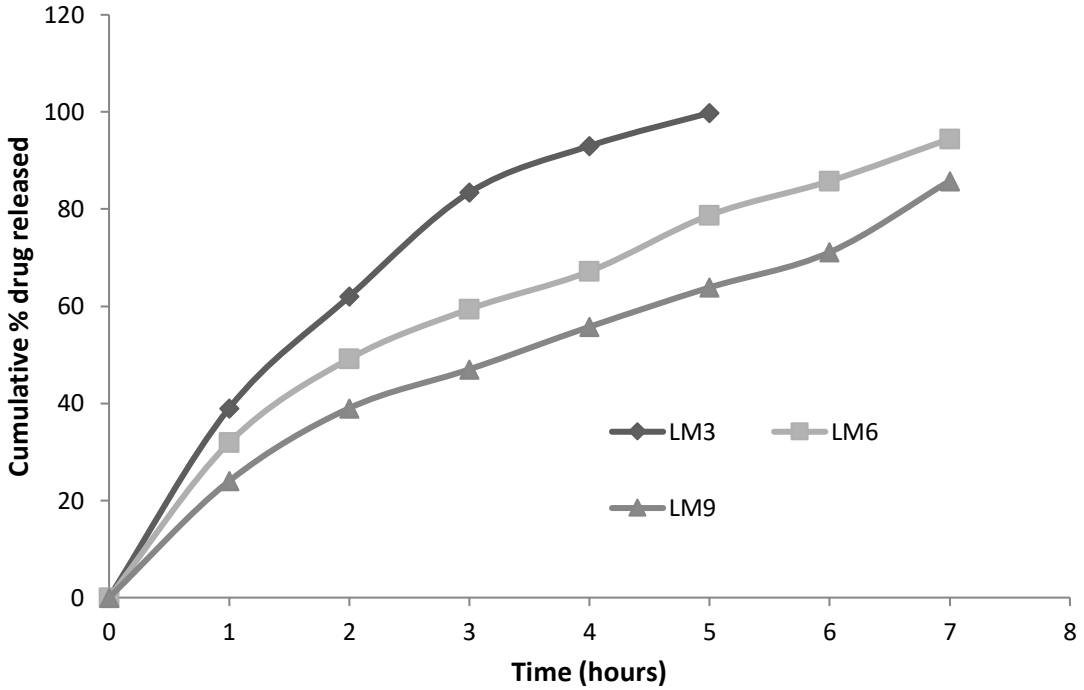

Figure 33: Effect of Different concentration of calcium chloride with 3 %w/w sodium alginate on drug release

Table 20: *In vitro* release kinetic data of Levofloxacin hemihydrate loaded mucoadhesive beads

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
LM1	18.9255	0.9912	-0.5741	0.8585	0.9947	---**	---**
LM2	16.5258	0.9954	-0.5312	0.7154	0.9937	---**	---**
LM3	12.9974	0.9923	-0.1821	0.9174	0.99424	0.6341	0.9992
LM4	17.33547	0.9964	-0.6928	0.7681	0.99245	---**	---**
LM5	14.3214	0.9958	-0.3855	0.7149	0.9931	0.5874	0.9994
LM6	13.4256	0.9987	-0.3041	0.7187	0.9981	0.6541	0.9941
LM7	19.6546	0.9911	-0.7147	0.7724	0.9961	---**	---**
LM8	11.3981	0.9842	-0.1627	0.9767	0.9957	0.5533	0.9964
LM9	09.8287	0.9931	-0.0987	0.9751	0.9951	0.5561	0.9928

* Insufficient data points to apply kinetics due to rapid release profiles

* *Insufficient data points to apply Korsmeyer-Peppas equation up to 70%.

K_0 – Zero order rate constant

K_1 – First order rate constant

R^2 – Regression coefficient

n- Diffusion exponent

ACCELERATED STABILITY STUDIES

Table 21: Accelerated stability data of Levofloxacin hemihydrate loaded mucoadhesive beads (Formulation F5)
[Tested according to ICH Q1A(R2)]

S.No	Time (days)	Mucoadhesive strength (Mean ± SE) (n = 3)	Drug content (%) (Mean ± SD) (n = 3)	Drug release(%) (Mean ± SD)(n =3)
1	Before storage (0 day)	69.55±0.55	25.85±0.43	99.81±0.27
2	30 days (After storage*)	68.98±0.42	25.41±0.81	99.41±0.25
3	90 days (After storage*)	67.91±0.86	25.18±0.52	99.31±0.11
4	180 days (After storage*)	67.03±0.63	25.01±0.41	98.84±0.58

*Storage at 40°C and 75% RH [n = 3].

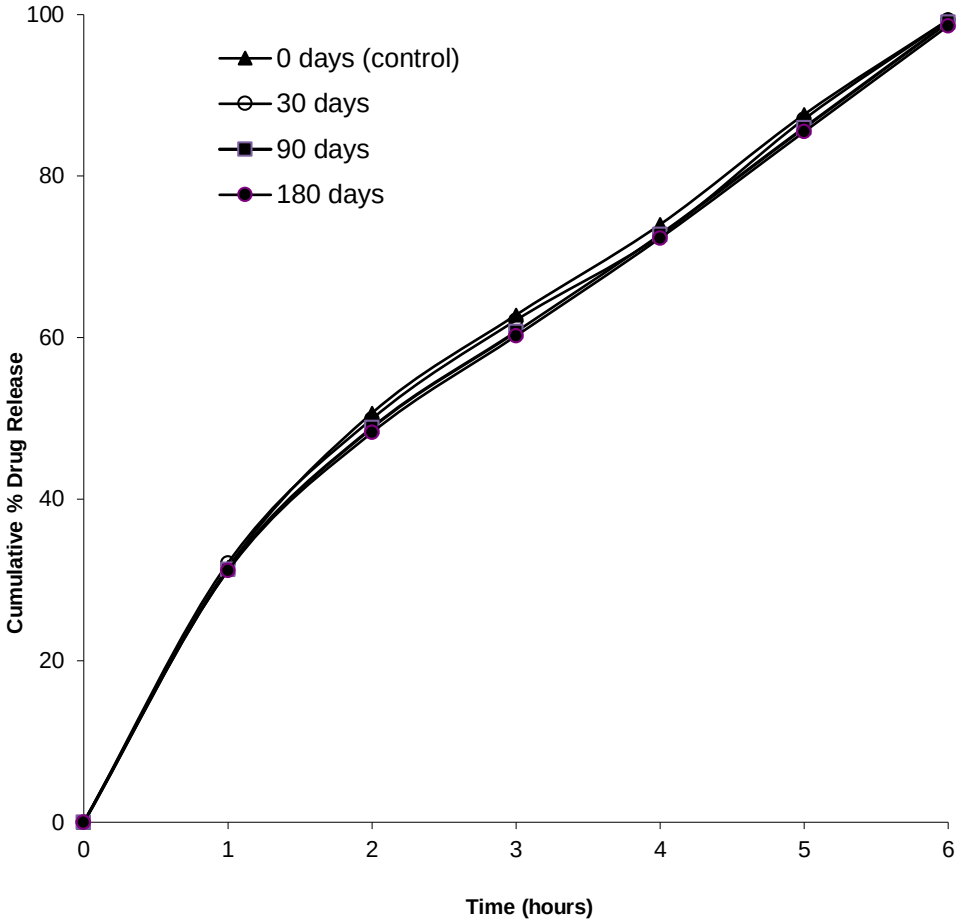

Figure 34: Stability study *in vitro* dissolution profile of Levofloxacin hemihydrate loaded beads in pH1.2 [Data points represents mean ± SD] [n=3].

10.DISCUSSION

In this study levofloxacin hemihydrate mucoadhesive beads were prepared by ion gelation method. Various concentration of sodium alginate and calcium chloride was used to investigate the effect of parameter on percentage yield, particle size, mucoadhesiveness, surface morphology of microspheres and drug release. Studies had revealed that mucoadhesive systems adhere to the mucosal membrane more strongly when the mucoadhesive polymer was in dispersed state.[92] In this study, mucoadhesive polymers were dispersed in the microspheres in order to enhance mucoadhesion. The standard curve of levofloxacin hemihydrate was plotted in 0.1 N HCl [pH 1.2] at 37^0C. It was found to be linear for the concentration ranges from 01 µgm/ml to 10 µgm/ml [R2 =0.9961] for levofloxacin hemihydrate [Table 4].

Percentage yield of microspheres

The percentage yield of levofloxacin hemihydrate loaded microspheres was shown in Table 5. The percentage yield for levofloxacin hemihydrate loaded microspheres were found to be in the range of 33.18±1.37% to 88.16±0.821%. The microspheres yield increased with increase in the concentration of sodium alginate and calcium chloride [$P < 0.05$]. It is evident from Table 5 that decreasing the polymer concentration has resulted in a decrease in the percentage yield. This effect can be explained by the fact that as the concentration of alginate decreases the quantity of polymer become insufficient to cover levofloxacin hemihydrate particles completely.

Drug content and encapsulation efficiency

The effects of polymer concentration on the drug content and encapsulation efficiency of the prepared microspheres were shown in Table 6 & 7. Drug content of the levofloxacin hemihydrate loaded microspheres varied from 20.58±0.82% to 29.90±0.61%. The encapsulation efficiency of the prepared microspheres varied from 52.31±0.23% to 79.55±0.55%. It was observed that drug loading was found to be directly proportional to polymer concentration. The encapsulation efficiency increased progressively by increasing the increase in the concentration of sodium alginate and calcium chloride [$P < 0.05$]. Higher loading efficiency was obtained as

the concentration of alginate increased. This may be attributed to the greater availability of active calcium binding sites in the polymeric chains and consequently, the greater degree of crosslinking as the quantity of sodium alginate increased.[176]

Particle size analysis- Shape and surface characterization

Viscosity of polymer solution is one of the most important factor related to formulation of beads. Flake formation was observed when low concentration of sodium alginate and calcium chloride was used at 1 % and 2%w/v, respectively, whereas maximum sphericity was observed at when high concentration of sodium alginate and calcium chloride was used at 3 % and 4 %w/v, respectively. The mean diameter of levofloxacin hemihydrate loaded microspheres were found to be in the range of 141.2±0.78 μm to 451.04±0.90μm [Table 9]. The results revealed that the increase 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 the research study which described that higher viscosity resulted from increase in the alginate concentration causes development of larger microspheres and greater drug entrapment due to high degree of crosslinking.[177] Researchers have suggested that as polymer concentration increases, the particle size also improves, which could be due to increase in the viscosity of drug and polymer ratio and thickness of polymer.[178]

The reason for the increased particle size with the higher polymer concentration is that the viscosity of the dispersed phase. Viscosity of dispersed phase increases with increasing polymer concentration. When the viscosity of the dispersed phase increases, it will be more difficult to break up the droplets to a smaller size and larger droplet will be formed. The beads prepared with different calcium chloride concentrations differed significantly in their shape. A similar increase in the size of beads was also observed with increase in calcium chloride concentration as well as cross-linking time. The addition of higher amount of calcium chloride will result in relatively more crosslinking of the guluronic acid units of sodium alginate, thereby leading to formation of larger beads.

***In vitro* evaluation of mucoadhesiveness**

The *in vitro* mucoadhesiveness study revealed that all the batches of prepared beads had good mucoadhesive property. Mucoadhesive property of the prepared microspheres varied from 46.52±0.57% to 84.51±0.74%. [Table 8]. A proportional increase in mucoadhesive strength of the formulation was observed with increase in the ratio of concentration of sodium alginate and calcium chloride.

It observed that mucoadhesion was higher when polymer concentration was achieved higher levels due to higher level formulations have more viscosity. Similarly, Nagda et al. also reported that as polymer concentration was increased, it leads to increased mucoadhesion.[179]

The following stages may have occurred during mucoadhesion. Initially, an intimate contact i:e [wetting] between the mucus gel and the swelling of mucoadhesive polymer. Which makes the polymer strands to relax, this is followed by the penetration of the mucoadhesive polymer into the mucus gel network and finally the formation of secondary chemical bonds between the mucus and the mucoadhesive polymer. The mucoadhesiveness of alginate is mainly related to the ability of carboxylic groups to form hydrogen-bonds with oligosaccharide chains of mucins.[180] Similarly, carboxyl groups in the polymer CP974P bind more strongly with the oligosaccharide chains of mucin owing to hydrogen bonding, and thus, mucoadhesive force is enhanced.[181,182] It was reported that that mucoadhesion was comparatively higher in acidic pH than that in intestinal pH. So, it is expected that the prepared formulation interacted greatly with the mucosubstrate on the surface of the stomach and adhered to the mucosa more strongly and could stay in the stomach for a prolonged period of time owing to higher anti *H. pylori* activity.[183] Maximum adhesion occurred when functional groups of polymer molecules were mostly in an undissociated state at pH < pKa.[184] The force required for detachment of particles from the mucus is much higher in lower acidic pH.[185] The polymer may adhere better at acidic pH [<pKa~5.9] levels where they are partly in a protonated condition and unionized state. Above pH > pKa, the carboxylic acid groups are ionized to a greater extent, thus reducing hydrogen bond formation.

Compatibility studies

DSC and FT-IR were performed on the raw materials and on the microspheres to detect interactions between the drug and the excipients.

FTIR studies

FTIR spectra were recorded for pure drug, drug-loaded microspheres and blank microspheres[Figure 10-12]. According to the FTIR analysis results, excipients and active substances did not interact. The FTIR spectra of pure levofloxacin hemihydrate showed characteristic peaks for –COOH monomeric stretching and bonding at 3269 and 1045 $^{cm-1}$, alkanes $–CH_3$ and aromatic rings 2846 and 1618 cm^{-1}, C=O stretching vibration of the COOH group 1721 cm^{-1} and C–F 835 cm^{-1}. All the above peaks peaks 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.

Differential Scanning Calorimeter [DSC] studies

DSC spectra were recorded for pure drug, drug-loaded microspheres and blank microspheres. The endothermic peak of levofloxacin hemihydrate is observed at about 237.3 ºC [Figure 13]. In the present study, the levofloxacin hemihydrate peaks were in agreement with the documented DSC chart in the literature under the same heating rate 10 C/min.[186] The DSC thermogram of the optimized formulation [LM5] showed a slight change in the peak shape with little broadening [Fig. 17] which could be attributed to the mixing process that lowers the purity of each component in the mixture.[187] The obtained thermograms indicated that there is no positive evidence for the inter action between levofloxacin hemihydrate and ingredients of the optimized formulation.

No endothermic peak corresponding to the levofloxacin hemihydrate was observed in levofloxacin hemihydrate loaded microspheres [Figure 17]. The absence of detectable crystalline domains in the microspheres clearly indicates that the drug was molecularly dispersed in the microspheres. The absence of detectable crystalline domains in the microspheres clearly indicates that drug was molecularly dispersed in the microspheres.

***In vitro* dissolution studies**

Dissolution test is a frequently used quality control method to evaluate drug release from oral dosage forms. Table 18 - 24 shows the data of drug release profiles of levofloxacin hemihydrate loaded beads of various formulations.

An initial burst effect was observed in all the batches of microsphere formulations, which may be due to the drug being adsorbed or located near the surface of the beads. However, as the polymer swelling proceeds, the remaining drug was released at a slower rate. This bi-phasic pattern of drug release was a distinguishing feature of matrix diffusion systems. The initial burst release effect was noticeably reduced with increase in polymer concentration. This might be because of increase in the density of the polymer matrix and also an increase in the diffusional path length, which the drug molecules have to traverse. Increasing concentration of the sodium alginate and calcium chloride, drug release was decreased it might be due to increased of thickness of gel diffusion layer.

A significant decrease in the rate and extent of drug release was observed with the increase in sodium alginate concentration in beads. The principal gelation or cross-linking of sodium alginate with calcium chloride is based on the tight junction between the guluronic acid residues.[188] The number of the apparent cross-linking points formed within increases with increasing alginate concentration in the formulation. This can be correlated with the particle size studies where, as alginate concentration increased, particle size increased, due to the formation of more a rigid and compact matrix, consequently retarding the drug release.

As the concentration of calcium chloride increased, drug release decreased. Beads prepared with 3% w/v calcium chloride showed the most sustained release effect due to more crosslinking resulting in the formation of a more rigid gel network and hence greater sustained release characteristics. It was also evident from the literature[188] that diffusion of drug from alginate matrix decreased as the concentration calcium chloride of solution increased, probably due to greater cross-linking with sodium alginate.

In vitro drug release and kinetics of release

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 R^2 value of 0.7149-0.9167 [Table20], whereas the same data, when plotted according to the zero order equation, improved the R^2 value of 0.9842-0.9987 and 0.9849-0.9986 [Table 18]. In our experiment, the *in vitro* release profiles of levofloxacin hemihydrate from all the formulations could at best be expressed by

Higuchi's equation, as the plots showed good linearity with R^2 value of 0.9901-0.9987 [Table 20]. 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 data were fit into the Korsmeyer *et al*,.[172] equation which showed high linearity with a comparatively high slope [n] value of 0.5533-0.6541 for levofloxacin hemihydrate loaded microspheres [Table 20]. This n-value indicated a coupling of diffusion and erosion mechanism. This type of drug release is called as anomalous diffusion. This indicates that drug release from the microspheres follows a non-Fickian trend. The presence of swelling polymers within the matrix structure might be responsible for the drug release controlled by more than one process. Thus, with regard to release kinetics, the data best fits in the Higuchi model and showed zero order release with a non-Fickian diffusion mechanism. The results showed that when an appropriate blend of these polymers was used, the drug release became more uniform in its kinetic approach towards zero order.

Selection of best formulation

The normal mucus turnover rate is 4–6 hours in rats and likely similar values in humans.[165] The mucus turnover rather than the mucus-polymer interaction that controls the presence of mucoadhesive formulations through the GIT. Based on the mucus turnover rate and dissolution time, formulations LM 5 [formulations consisting of 2% w/v Sodium alginate, 1% w/v carbopol 974P and 3% w/v Calcium chloride] were selected as best formulations. Accelerated stability studies were conducted for formulation LM 5.

High concentration of matrix polymer solution increases viscosity of the polymer droplet and delays the drug diffusion within the polymer droplets. Larger microspheres have a smaller total surface area per unit mass and, consequently, a lower amount of drugs diffuses in to liquid paraffin. As a result drug content of the larger microspheres was higher.[188]

Accelerated stability testing according to ICH Q1A [R2]

The optimized formulation [LM 5] were stored in a stability chamber [Remi CHM- 10 S®, India] at 40 ± 2°C and at a humidity of 75 ± 5% RH for 6 months and

observed for the drug content, mucoadhesiveness and *in vitro* drug release on 0, 30, 90, and 180 days [Table 21]. 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 [Figure 34].

11. CONCLUSION

H. pylori colonize the gastric mucosa leading to gastritis, gastric ulcer, and gastric carcinoma. To increase the efficacy of eradicating the infection, a localized delivery system of anti-H. *pylori* agents in the stomach is required. Mucoadhesive beads of levofloxacin were prepared to increase the local concentration of the antibiotic in the stomach to eradicate *H. pylori* infection. The main goal of this study was to optimize mucoadhesiveness and controlled release property. The optimized formulation for levofloxacin mucoadhesive beads was obtained with Sodium alginate, Carbopol 934P and calcium chloride. Based on the mucus turnover rate and dissolution time, formulations LM 5 (formulations consisting of 2% w/v Sodium alginate, 1% w/v carbopol 974P and 3% w/v Calcium chloride) were selected as best formulations *Invitro* studies clearly indicates that the prepared formulations possess good bioadhesive properties. These properties enable the microspheres to adhere to the gastric mucosal surface and stay in stomach for prolonged periods, which eventually resulted in better eradication of *H. pylori* than the conventional dosage forms. This study proves the possibility of delivering levofloxacin for the treatment of *H. pylori* infection.

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