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Formulation and *in-vitro* characterization of floating mucoadhesive beads of Levofloxacin Hemihydrate

Rukmangathen Rajalakshmi*, Chimmiri Padmaja, Yerram Chandra Mouli, Amaravathi Vikram, Balambhaigari Rubia Yasmeen and Velam Vinesha

Department of Pharmaceutics, Sree Vidyankethan College of Pharmacy, A. Rangampet, Tirupati, India

ABSTRACT

The main aim of this investigation was to develop gastroretentive beads for controlled release of the drug for the treatment of *H.pylori* infections more efficiently by releasing the drug especially in stomach for a prolonged duration of time. Floating mucoadhesive beads were prepared to prolong the gastric retention of the drug. Floating mucoadhesive alginate - hydroxypropyl methylcellulose beads of Levofloxacin hemihydrate were prepared by using emulsion gelation method. The interactions between drug and polymers were investigated by Fourier transform infrared (FTIR) Spectroscopy and Differential scanning calorimetry (DSC). Prepared beads were evaluated for particle size, entrapment efficiency, and surface morphology by using scanning electron microscopy. *In vitro* drug release studies were carried out. According to FTIR and DSC, the drug did not show any evidence of an interaction with the polymers used. From the *in vitro* drug release studies, the drug release from formulation F6 was found to be 71.68 % and showed controlled release. From the results it can be concluded that F6 formulation was found to be better than the other formulations, as it showed controlled drug release. Hence it may achieve the aim of controlling the drug release, prolong retention time in GIT and reduce the frequency of dosing.

Key words: Emulsion gelation method, Entrapment efficiency, Gastric retention, *H.pylori* infections, alginate-hydroxypropyl methyl cellulose beads

INTRODUCTION

There are various types of Controlled Drug Delivery System, currently available in the market, but the focus of this research is the stomach specific drug delivery system, which is the floating drug delivery system (FDDS). The FDDS uses the gastro-retentive technique for drugs which are absorbed from the stomach and is poorly absorbed or insoluble in the intestine due to the high pH environment. It is known that for drugs to be absorbed across the plasma membrane, it should not be ionised and to be remained hydrophobic for effective absorption. The pH environment of the stomach is acidic and cleared of food periodically according to the unpredictable gastric emptying time (GET) and gastric residence time (GRT) [1-3]. In order to overcome such physiological adversities, one such approach being used is the FDDS [4].

FDDS is one of the gastro retentive drug delivery system to achieve gastric retention to obtain sufficient drug bioavailability. These systems are less density than the gastric fluids and make buoyant, release the drug slowly as a desired rate from the system. The concept of floating system suffers from a disadvantage that it is effective only when the fluid level in stomach is sufficiently high. As the stomach empties, the dosage form is at the pylorus, the

buoyancy of the dosage form may be impeded [4]. This limitation can be overcome by the use of bio-adhesive polymers to enable it to adhere to the mucous lining of stomach wall.

Bioadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio, a much more intimate contact with the mucus layer, and specific targeting of drugs to the absorption site [5-8]. Floating and bio-adhesive drug delivery systems offer the advantages of increased contact time with stomach mucosa, provides more effective absorption and bioavailability of drugs with absorption windows near proximal intestine and stomach, thus dosing frequencies can be decreased [9]. Therefore, it is easy to reach minimum inhibitory concentration in the gastric mucosa where *H. pylori* colonize [10].

Levofloxacin hemihydrate is used as anti microbial agent for the treatment of variety of infectious diseases. It is safe and effective in first, second, and third line *H.pylori* eradication. Eradication rate was 90% for the Levofloxacin therapy. The antimicrobial action of Levofloxacin hemihydrate results from inhibition DNA gyrase and topoisomerase IV essential enzymes involved in the replication, transcription and repair of bacterial DNA [11].

The main objective of this research work was to develop and evaluate the floating mucoadhesive beads of Levofloxacin hemihydrate to prolong gastric residence time and to increase its bioavailability. The dose of Levofloxacin is 200-400mg. To reduce the frequency of dosing, floating mucoadhesive beads have been prepared. Floating mucoadhesive beads were prepared using sodium alginate, HPMC K₄M and liquid paraffin by emulsion gelation method. Thus the study aims to improve the oral bioavailability of the drug and to achieve extended retention in the stomach which may result in prolonged absorption.

MATERIALS AND METHODS

Materials

Levofloxacin hemihydrate and HPMC K₄M were obtained as a gift sample from MMC healthcare limited and Fine chemicals limited respectively. Sodium alginate purchased from Burgoyne urbidges & co. Calcium chloride (CaCl₂) and Light liquid paraffin are obtained from Hi-media labs, Mumbai. All other ingredients used were of analytical grade.

Preparation of beads

Floating Mucoadhesive beads of Levofloxacin hemihydrate were prepared by emulsion gelation method. In this present work six formulations of floating mucoadhesive beads were prepared using sodium alginate, HPMC K₄M and liquid paraffin in different combinations. The composition of different formulations of floating mucoadhesive beads of Levofloxacin hemihydrate were mentioned in Table 1.

Table 1: Formulation of floating mucoadhesive beads of Levofloxacin hemihydrates

S. No	Formulation	Levofloxacin hemihydrate(mg)	Sodium Alginate(g)	Liquid paraffin (%w/w)	HPMC K ₄ M (g)	CaCl ₂
1	F1	500	1.5	-	-	2%
2	F2	500	1.5	-	0.5	2%
3	F3	500	1.5	5	-	2%
4	F4	500	1.5	10	-	2%
5	F5	500	1.5	15	-	2%
6	F6	500	1.5	5	0.5	2%

Sodium alginate and HPMC K₄M at specified ratio were dissolved in 50ml of distilled water to form homogenous polymer solution. Levofloxacin hemihydrate was added to polymer solution and mixed thoroughly to form a viscous dispersion. Then liquid paraffin was added to this dispersion and mixed thoroughly. Resulting uniform dispersion was added drop wise in to 2% CaCl₂ solution through syringe fitted with a needle of 24 gauge. Spherical rigid beads were formed in CaCl₂ solution. Beads were collected by decanting the supernatant liquid and product thus formed was washed repeatedly with water and dried at room temperature overnight and stored in desiccator for further use [12].

Compatibility studies**Fourier Transform Infrared Spectroscopy (FTIR)**

The interaction between the drug and polymers was studied by IR peak matching technique. The drug and polymer were taken and mixed uniformly with demohydrated KBr. The mixture was compressed to a thin transparent pellet by subjecting to hydraulic press, which is placed in the path of IR rays using a sample holder to record the spectra from 400 – 4000 Cm^{-1} .

Differential Scanning Calorimetry

Thermal analysis of Levofloxacin hemihydrate, sodium alginate, HPMC K₄M and physical mixture were recorded with Netzsch DSC 200PC (Netzsch, Selb, Germany). The temperature axis and cell constant of DSC were previously calibrated with Indium. A heating rate of 5°/min was employed over a temperature range of 0- 350° with nitrogen purging. Powder sample was weighed into an aluminium pan was used as reference.

Evaluation Studies**Yield of floating mucoadhesive beads**

The yield of the beads was expressed as percentage of the weight of the dried beads at room temperature compared to the theoretical amount. Percentage of yield was calculated by using the Equation:

$$\text{Percentage yield} = \frac{\text{The amount of beads obtained (g)}}{\text{Theoretical amount (g)}} \times 100$$

Size analysis of floating beads

The mean diameter of 100 dried beads was determined by optical microscopy (Metzer, India). The optical microscope was fitted with a stage micrometer by which the size of beads was determined.

Micromeritics**Bulk Density**

The bulk density is defined as the mass of powder divided by bulk volume. The bulk density was calculated by dividing the weight of the samples in grams by the final volume in cm [12].

$$\text{Bulk Density} = \frac{\text{Mass of the beads}}{\text{Bulk volume of the beads}}$$

Tapped Density

Tapped density is the volume of powder determined by tapping the weighed amount of sample in measuring cylinder. The cylinder containing known amount of beads was tapped on a tapped density apparatus until it gives constant volume [13].

$$\text{Tapped Density} = \frac{\text{Mass of the beads}}{\text{Tapped volume of the beads}}$$

Carr's Index or Compressibility Index

This is an important property in maintaining uniform weight. It was calculated using following equation:

$$\% \text{ compressibility index} = 1 - \frac{\text{Bulk density}}{\text{Tapped density}} \times 100$$

Hausner's ratio

A similar index like percentage compressibility index has been defined by Hausner. Values less than 1.25 indicate good flow, where as greater than 1.25 indicates poor flow. Flow of the material under study was normally improved by addition of glidant. Hausner's ratio calculated by formula:

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}} \times 100$$

Angle of Repose (θ)

Inter particle forces between particles as well as flow characteristics of powders were evaluated by angle of repose. Angle of repose is defined as the maximum angle possible between the surface and the horizontal plane. The angle of repose of powder blend was determined by glass funnel method. Powders were weighed accurately and passed freely through the funnel, so as to form a heap. The height of funnel was so adjusted that the tip of the funnel just touched the apex of the heap. The diameter of the powder cone so formed was measured and the angle of repose was calculated using the following equation [13]:

$$\theta = \tan^{-1}\left(\frac{h}{r}\right)$$

Where, θ = angle of repose

h = height of the pile and,

r = radius of the powder cone respectively.

Scanning Electron Microscopy (SEM)

The SEM analysis of prepared beads was performed for morphological studies. The formulations are poured in to circular aluminum stubs using double adhesive tape, and coated with gold in HUS -5GB vacuum evaporator, and observed in Hitachi S-3000N SEM at an acceleration voltage of 10 Kv and a magnification of 5000X.

Floating behavior

300 mg of the dried beads were spread over the surface of a USP dissolution apparatus Type II using Simulated gastric fluid without enzymes of pH 1.2 was used as medium (900 ml) and was maintained at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 12 hrs. The paddle speed was controlled at 100 rpm. The floating and the settled portion of beads were recovered separately. After drying, each fraction of the beads was weighed and their buoyancy was calculated by the following equation [14]:

$$\% \text{ Buoyancy} = \frac{Q_f}{Q_f + Q_s}$$

Q_f = Weight of beads floating on the surface

Q_s = Weight of beads sinked

Drug entrapment efficiency

The amount of drug entrapped was calculated by taking the formulation equivalent to 50 mg of the drug. The amount of drug entrapped was estimated by crushing the beads and extracting with aliquots of 0.1 N HCl, repeatedly. The extract was transferred to a 100 ml volumetric flask and the volume was made up using 0.1 N HCl. The solution was filtered and the absorbance was measured at 293 nm against 0.1 N HCl as blank [15, 16]. The amount of drug entrapped in the beads was calculated by the following formula:

$$\text{Amount of drug entrapped} = \frac{\text{Amount of the drug actually present}}{\text{theoretical drug load expected}} \times 100$$

In vitro test for mucoadhesion

The time taken for detachment of beads from stomach mucosa was measured in 0.1N hydrochloric acid (pH 1.2). This was evaluated by an *in vitro* adhesion testing method, known as wash off method. A piece of sheep stomach mucosa (2×2 cm) was mounted onto glass slide with cyanoacrylate glue and one more glass slide was connected with a support. 50 beads were counted and spread over the wet rinsed tissue specimen and immediately thereafter the support was hung on the arm of a USP tablet disintegrating test machine. By operating the disintegration machine the tissue specimen was given a slow regular up and down moment. The slides move up and down in the test fluid at $37 \pm 0.5^{\circ}\text{C}$. The number of beads adhering to the tissue was counted at two hours interval up to 6 hours [17, 18].

In vitro drug release study

In vitro drug release studies were carried out by using USP type I dissolution test apparatus using 0.1 N HCl as dissolution medium, maintained at $37 \pm 0.5^{\circ}\text{C}$ and stirred at 50 rpm. Beads equivalent to 100 mg of the drug were

taken. 5ml of sample aliquot was withdrawn at predetermined intervals and filtered. Equal volume of the dissolution medium was replaced in the vessel after each withdrawal to maintain sink condition. The required dilutions were made with same medium and the solutions were analyzed for the drug release by spectrophotometer at 293. From this, the percentage of drug release was calculated and plotted against function of time to study the pattern of drug release [14].

RESULTS AND DISCUSSION

Compatibility studies

FT IR studies

IR spectra were recorded for the pure drug and drug loaded beads and shown in Figure 1. The results of FTIR spectra confirm that there were no interactions between drug and polymer. Four bands characteristic of O-H stretching in carboxylic acid, C=C bending, Aryl fluoride and C=O stretching of the pure drug was unchanged in the prepared formulation.

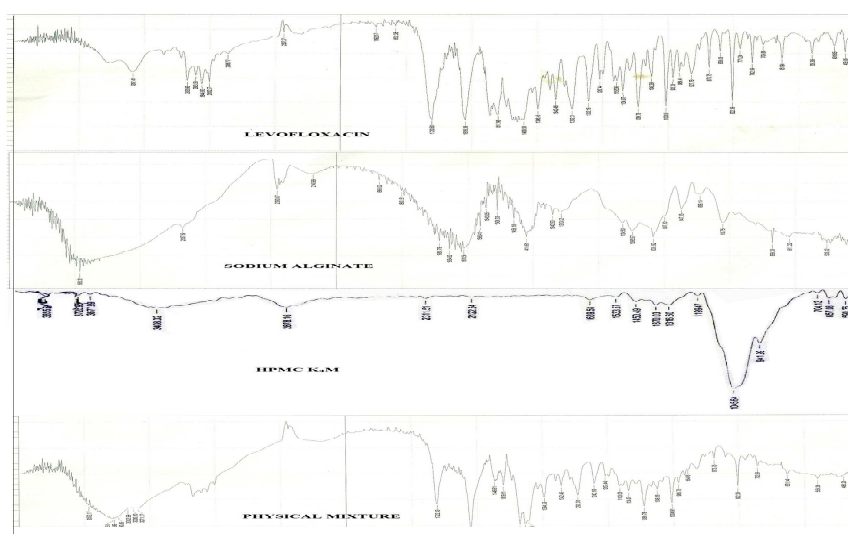


Figure 1: FTIR spectra of Levofloxacin hemihydrate, sodium alginate, HPMC K4M and physical mixture

DSC studies

From DSC thermograms the melting point of pure drug Levofloxacin hemihydrate was found to be 223 °C, which is close to the value reported in literature hence the procured drug is pure form (Figure 2). The physical mixture DSC thermograms indicate that there are no interactions between the drugs and excipients which can be accessed from the peaks in the DSC thermograms.

Evaluation Studies

Particle Size

Particle size was determined by using optical microscopy. The mean particle size (mean diameter) was in range of 0.59 mm to 1.254 mm (Figure 3 and Table 2). The mean particle size of the floating beads was increased as the concentration of oil increases. It suggests that the as the concentration of oil increases the amount of oil entrapped in floating beads was increased.

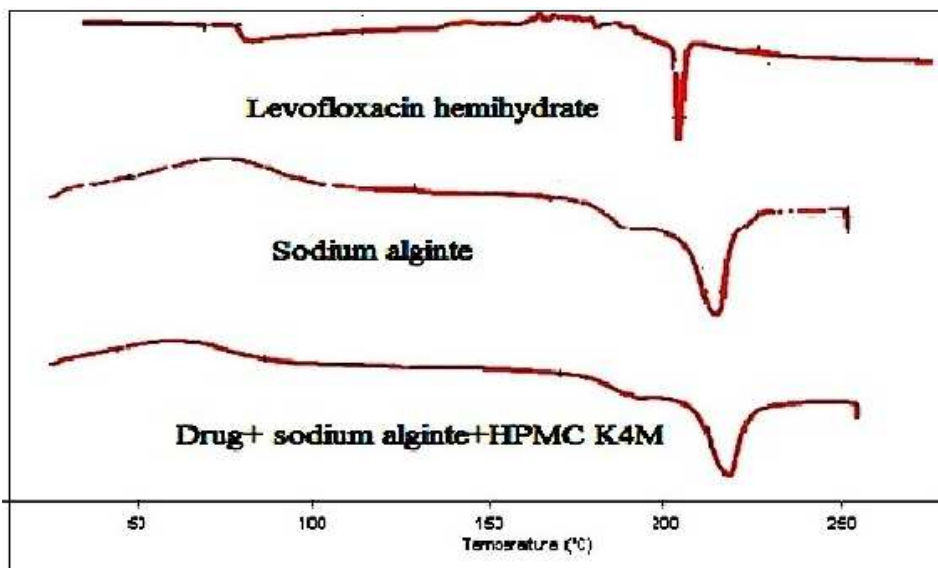


Figure 2: DSC thermograms of Levofloxacin hemihydrate, sodium alginate and Physical mixture

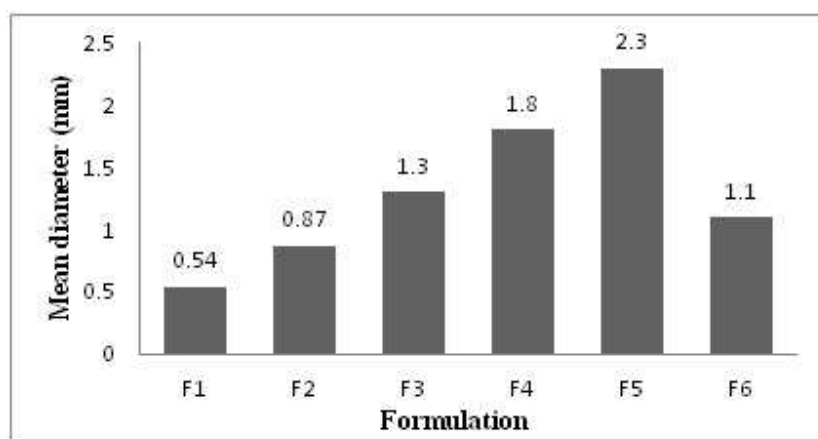


Figure 3: Particle size distributions of various formulations of Levofloxacin hemihydrate floating Mucoadhesive beads

Micromeritic Properties

Results of the bulk and tapped densities, angle of repose, Carr's index (compressibility index), and Hausner's ratio of all beads confirms better flow properties, values were reported in the Table 2

The angle of repose (θ) is a characteristic of the internal friction or cohesion of the particles. The value of the angle of repose will be high for cohesive powder and low for non-cohesive powder. The prepared formulations of Levofloxacin hemihydrate showed values θ in 20-27 $^\circ$ indicates that they had better flow property. Carr's index up to 21 is considered of acceptable flow properties. Hausner's ratio was related to the inter particle friction, the powders with low inter particle friction, had ratios of approximately 1.25 indicating good flow.

Table 2: Flow properties and mean diameter of various formulations of Levofloxacin hemihydrate floating mucoadhesive beads of Levofloxacin hemihydrate

Formulation	Bulk Density(g/cm ³)	Tapped Density(g/cm ³)	Carr's index (%)	Hausner's ratio	Angle of repose(θ)	Mean diameter (mm)
F1	0.403	0.469	14.19	1.14	25 ⁰ 20'	0.54
F2	0.341	0.401	15.04	1.17	21 ⁰ 25'	0.87
F3	0.440	0.521	15.47	1.18	27 ⁰ 89'	1.3
F4	0.400	0.468	14.53	1.16	23 ⁰ 96'	1.8
F5	0.318	0.364	12.32	1.13	20 ⁰ 22'	2.3
F6	0.412	0.486	15.07	1.17	27 ⁰ 75'	1.1

Surface morphology

F2, F3, F5 of the prepared beads were evaluated for the surface morphology and shown in the Figure 4. Scanning electron microscopy revealed that the prepared beads were spherical and the surface of the beads was porous and rough. The porous nature of the microcarriers increases the floating behavior of the beads. The results of the SEM suggest that upon increasing the oil concentration the shape of the beads somewhat irregular.

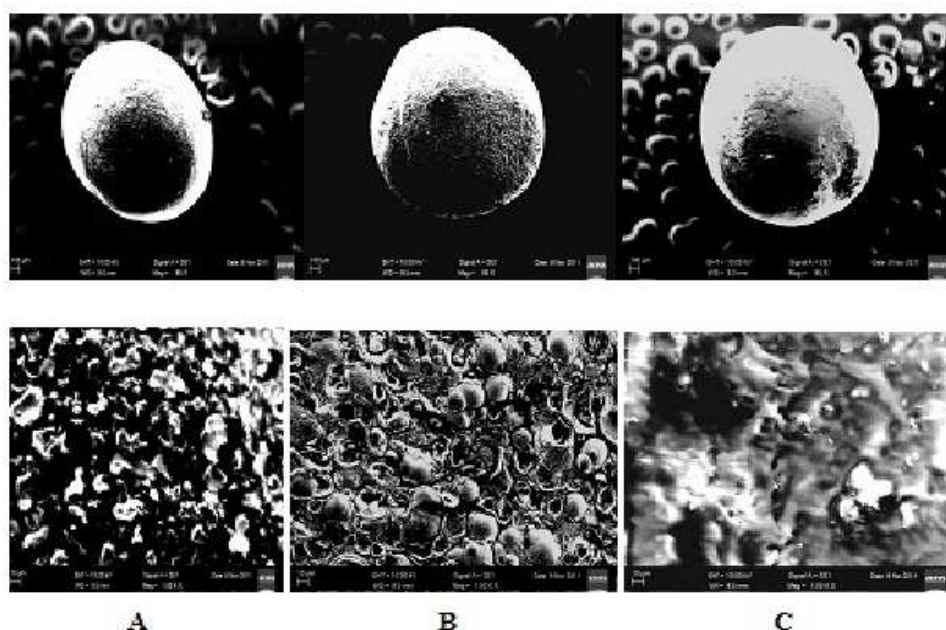


Figure 4: SEM photographs of various formulations of Levofloxacin hemihydrate floating mucoadhesive beads

Floating behaviour

The concentration of entrapped oil influences both buoyancy lag time and the % buoyancy. As the concentration of oil increases, the buoyancy lag time decreases because the entrapped oil content was more. The formulation containing 15% of liquid paraffin (F5) showed 100 % buoyancy and the formulations which do not containing liquid paraffin (F1 and F2) showed 0% buoyancy. Floating ability of the beads depends upon the amount of the liquid paraffin used in the preparation. The results were given in Table 3.

Entrapment efficiency

% Entrapment efficiency of the floating mucoadhesive beads in 0.1N HCl was calculated and results were mentioned in Table 3. The % Entrapment efficiency of the formulations was found to be in the range of 72.22 – 95.06%. Formulation that contains both HPMC K₄M and sodium alginate (F2 and F6) showed highest % of entrapment because of the polymers used had high entrapment efficiency. The formulation containing 15% of liquid paraffin (F5) showed less % of entrapment because increase in oil percentage decreases the space for the entrapment of the drug within the beads.

Table 3: % of Buoyancy and % drug entrapment of various formulations of Levofloxacin hemihydrate floating mucoadhesive beads

Formulation	Buoyancy lag time	% Buoyancy	% Drug entrapment
F1	-	0	94.12
F2	-	0	95.06
F3	30 sec-1 min	87.37	85.58
F4	30 sec-1 min	90.45	79.83
F5	0-30 sec	100	72.22
F6	30 sec-1 min	78.65	87.31

***In vitro* mucoadhesion test**

% mucoadhesion was calculated and mentioned in Table 4. The formulations containing HPMC K₄M (F2 and F6) showed highest mucoadhesion than the formulations without the polymer. The hydrophilic residues of HPMC K₄M can bind with water at the surface and within the gel. Initially, an intimate contact that is wetting occurs between the mucus and the mucoadhesive polymer, which is followed by the penetration of the mucoadhesive polymer into the mucus gel network. Finally the formation of secondary chemical bonds between the mucus and the mucoadhesive polymer occurs. The chains can diffuse into the mucosal layer and remain adhered for long periods.

Table 4: % Mucoadhesion of various formulations of Levofloxacin hemihydrate floating mucoadhesive beads

Formulation	% Mucoadhesion at different time intervals		
	2hr	4hr	6hr
F1	68	56	52
F2	94	88	86
F3	60	54	50
F4	66	58	54
F5	62	56	52
F6	92	86	84

***In vitro* drug release**

The release pattern of drug from formulations prepared with combination of polymers i.e. with sodium alginate and HPMC K₄M was studied. The results were showed in Table 5 and Figure 5. The formulation F6 showed controlled release than other formulations. It is observed that as the concentration of oil increases, the drug release was reduced. It is due to the oil acts as additional barrier for the drug release. The controlled release of the drug from the beads was due to the formation of thick layer of polymer which would hold the drug with itself. The concentration of oil increases from F3 – F5 and the % drug release was found to be decreased. Finally it was observed that the formulations prepared with oil showed better controlled release than formulations F1 & F2.

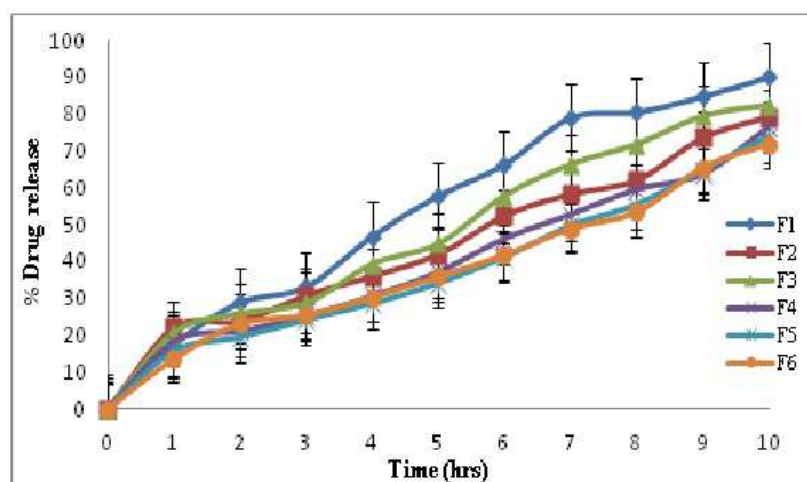


Figure 5: Graph showing cumulative % drug release of various formulations of Levofloxacin hemihydrate floating mucoadhesive microspheres

CONCLUSION

Oral controlled release of Levofloxacin hemihydrate can be achieved successfully by beads prepared by Emulsion gelation technique using sodium alginate, HPMC K₄M and liquid paraffin. The oil-entrapped beads showed excellent, immediate buoyancy until the stomach contents are full after that the mucoadhesive polymer HPMC K₄M increases the gastric residence by its mucoadhesive property. Therefore this formulation provide a suitable manner to deliver drugs that are locally active to the gastric mucosa in the stomach and, hence, achieve a sustained site-specific therapeutic action for *Helicobacter pylori* eradication in the treatment of peptic ulcer disease.

Sodium alginate HPMC K₄M beads may be more suitable floating-mucoadhesive drug delivery system for delivering Levofloxacin hemihydrate to treat stomach ulcers compared with beads which do not contain HPMC K₄M.

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