Design and Characterization of Polymeric Floating Microspheres of Levofloxacin Hemihydrate.

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The complex of Levofloxacin and Chitosan lead to formation of water soluble complex. This complex of Levofloxacin was then formulated into floating beads calcium alginate with maximum entrapment of Levofloxacin found to be about 75 %(f10) and the entrapment was found to be significantly higher as compared to the other formulations (f1 to f9). *In- Vitro* Release studies of the beads f10 was found to significantly improve the release of Levofloxacin as compared to other formulations. The mean particle size of f10 microspheres and surface morphology were determined by SEM Resulting in Porous and Rough Surface of microspheres. The drug release kinetics were studied as zero order, first order , Higuchi , Koresmeyer-Peppas equations , good linearity was found in Higuchi's Equation (R^2= 0.9310) indicating the release of the drug from Microspheres is based on Fickian Diffusion .

Key words: Levofloxacin, Chitosan, Calcium Alginate, Microspheres, release kinetics, SEM, Fickian Diffusion

1. Introduction

Floating drug delivery systems (FDDS) or hydro dynamically controlled systems are low-density systems that have sufficient buoyancy to float over the gastric contents and remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time. While the system is floating on the gastric contents, the drug is released slowly at the desired rate from the system. After release of drug, the residual system is emptied from the stomach. This results in an increased Gastric retention time and a better control of the fluctuations in plasma drug concentration. However, besides a minimal gastric systems have been developed based on beads , granules, powders, capsules, tablets, laminated films and hollow microspheres content needed to allow the proper achievement of the buoyancy retention principle, a minimal level of floating force (F) is also required to keep the dosage form reliably buoyant on the surface of the meal Many buoyant.[1-5]

While the system is floating on the gastric contents, the drug is released slowly at the desired rate from the system. After release of drug, the residual system is emptied from the stomach. This results in an increased Gastric retention time and a better control of the fluctuations in plasma drug concentration. However, besides a minimal gastric content needed to allow the proper achievement of the buoyancy retention principle, a minimal level of floating force (F) is also required to keep the dosage form reliably buoyant on the surface of the meal Many buoyant systems have been developed based on beads , granules, powders, capsules, tablets, laminated films and hollow microspheres the drugs that is majorly absorbed from stomach or proximal part of the intestine. The pattern of motility is however distinct in the two states. During the fasting state an interdigestive series of electrical events take place, which cycle both through stomach and intestine every 2 to 3 hours. This is called the interdigestive myloelectric cycle or migrating myloelectric cycle (MMC), which are further divided into following 4 phases are:

1. Phase I (basal phase) It lasts from 40 to 60 minutes with rare contractions.
2. Phase II (preburst phase) It lasts for 40 to 60 minutes with intermittent action potential and contractions. As the phase progresses the intensity and frequency also increases gradually.
3. Phase III (burst phase) lasts for 4 to 6 minutes. It includes intense and regular contractions for short period. It is due to this wave that all the undigested material is swept out of the stomach down to the small intestine. It is also known as the housekeeper wave.
4. Phase IV It is a period of transition from phase III and phase I and last for 0 to 5 minutes. [5-7]
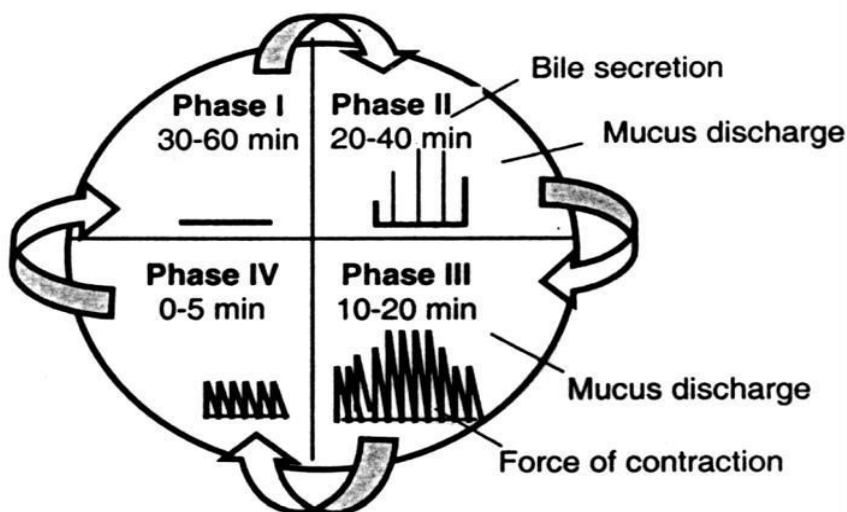

Figure 1.Interdigestive myloelectric cycle or migrating myloelectric cycle (MMC).

Mechanism of floating systems

Various attempts have been made to retain the dosage form in the stomach as a way of increasing the retention time. These attempts include introducing floating dosage forms

1. Gas-generating systems
2. Swelling or expanding
3. Mucoadhesive systems
4. High-density systems
5. Low density system

Floating drug delivery systems (FDDS) have a bulk density less than gastric fluids and so remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time. The drug is released slowly at the desired rate from the system. After release of drug, the residual system is emptied from the stomach. This results in an increased GRT and a better control of the fluctuations in plasma drug concentration. However, besides a minimal gastric content needed to allow the proper achievement of the buoyancy retention principle, a minimal level of floating force (F) is also required to keep the dosage form reliably buoyant on the surface of the meal. To measure the floating force kinetics, a novel apparatus for determination of resultant weight has been reported in the literature. The apparatus operates by measuring continuously the force equivalent to F (as a function of time) that is required to maintain the submerged object. The object floats better if F is on the higher positive side. This apparatus helps in optimizing FDDS with respect to stability and durability of floating forces produced in order to prevent the drawbacks of unforeseeable intragastric buoyancy capability variations.[8-21]

$$F = F \text{ (buoyancy)} - F \text{ (gravity)}$$
$$= (Df - Ds) \, gv \quad \text{--- (1)}$$

Where, F= total vertical force,

Df = fluid density,

Ds = object density,

v = volume of GI fluid and

g = acceleration due to gravity

1.1 Levofloxacin Hemihydrate

Levofloxacin and other fluoroquinolones are valued for their broad spectrum of activity, excellent tissue penetration, and for their availability in both oral and intravenous formulations. Levofloxacin is used alone or in combination with other antibacterial drugs to treat certain bacterial infections including pneumonia, urinary tract infections, and abdominal infections. Levofloxacin and other fluoroquinolones are generally well tolerated, but in rare instances have produced serious adverse reactions such as spontaneous tendon ruptures and irreversible peripheral neuropathy.

2. Methods:

Standard Levofloxacin Hemihydrate LR and polymer Chitosan purchased form Research-Lab Fine Chemical Industries and Sodium Alginate, sodium bicarbonate, calcium carbonate Procured from LOBA Chemie Pvt .Ltd. Mumbai

1. Preparation and Optimization of Levofloxacin and Chitosan complex

Levofloxacin was mixed with Chitosan in different proportions and the optimum proportion of the drug and the polymer was established to get the water soluble complex. UV Spectrophotometer for solubility analysis of the maximum Levofloxacin dissolves in water in presence of Chitosan. All the floating beads formulation of Levofloxacin dissolves in water. Wavelength scanning from 400 nm to 200 nm. Peaks should be shows at a 288 nm of the all floating beads formulation Chitosan in different ratios they as following. The soluble complex of Levofloxacin and Chitosan was then utilized with various Proportions of Sodium alginate, Sodium bicarbonate and calcium carbonate to get Maximum entrapment of Levofloxacin in the floating beads. Factorial design was applied to design the experiment and optimization of maximum entrapment of Levofloxacin in Microspheres.

2. Preparation of calcium alginate Levofloxacin Floating Microspheres

Aqueous solution of Sodium alginate solution (2% 2g/100ml of water w/v) was prepared by suspending 2 gm of Sodium alginate in 100 ml of distilled water. The suspension was kept under continuous stirring using magnetic stirrer till homogenous solution was obtained. Complex of Levofloxacin with Chitosan was prepared by mixing 1.0 gm of Levofloxacin and concentration various formulation chitosan . The complex (52gm) was then mixed with 1.0gm sodium bicarbonate and 1.6gm of calcium carbonate . The mixture of levofloxacin , chitosan, Sodium bicarbonate and Calcium carbonate obtained in step "b" was then added to 50ml of Sodium alginate solution and stirred thoroughly to get uniformly suspended mixture. Dissolved 2 gm of Calcium chloride in about 80 ml of distilled water and made up the volume to 100 ml to get 2%w/v solution of Calcium chloride. The floating beads of Levofloxacin we prepared by adding drop wise the mixture obtained in step "c" under stirring condition. The beads obtained in step were separated by filtration and weighed. The Wet beads were then dried at 40º C for 2 hrs. The beads were then kept at room temperature for overnight for further dried to form microspheres. (Table 1.0)

3. Encapsulation Efficiency

 1 g of Levofloxacin, weight of dried CLFB different formulation of Levofloxacin determination of following parameter for drug loading and encapsulation efficiency.

  a) **% drug loading   (DL)** = Weight of Levofloxacin in beads/weight of beads*100
  b) **% theoretical   loading (TL)** = Weight of Levofloxacin added/Weight of Levofloxacin added + Weight of Polymer added × 100
  c) **% entrapment efficiency (EE)** = % Drug Loading / % Theoretical Loading × 100

Formulation / Content	LV (gm)	CH (gm)	CC (gm)	SB (gm)	CA (gm)	SA (gm)
F1	1.0gm	—	0.5gm	1.0gm	2%	2%
F2	1.0gm	—	1.0gm	1.0gm	2%	2%
F3	1.0gm	0.5gm	0.5gm	1.0gm	2%	2%
F4	1.0gm	0.5gm	1.0gm	—	2%	2%
F5	1.0gm	—	1.0gm	—	2%	2%
F6	1.0gm	1.0gm	1.0gm	1.0gm	2%	2%
F7	1.0gm	—	—	1.0gm	2%	2%
F8	1.0gm	0.5gm	0.5gm	0.5gm	2%	2%
F9	1.0gm	0.5gm	1.0gm	0.5gm	2%	2%
F 10	1.0gm	1.0gm	1.0gm	0.5gm	2%	2%
F11	1.0gm	—	0.5gm	0.5gm	2%	2%

Table 1.0 Formulation of Floating Microspheres with composition of different excipients

LV = Levofloxacin , C.C. = calcium carbonate, S.B. = sodium bicarbonate, CH = Chitosan, S.A. = Sodium alginate solution 2 %, CA = Calcium chloride solution 2%

4. DSC and TGA analysis
Differential Scanning Calorimetry and Thermal Gravimetry Analysis for Sodium Alginate Levofloxacin Hemihydrate Floating Microspheres Formulation F10- melting point of standard Levofloxacin is 215C^O. Scanning range is 30 to 320^0 with respect to heating rate 10^{0}C per minutes by Pyris series DSC 6000.

5. FT-IR Study
Drug and Drug polymer interaction were studied by FT-IR Spectroscopy. The infrared Spectra of levofloxacin and Drug loaded Microspheres were recorded on FT-IR (sican2301).The sample were prepared on KBr press and the spectera Were recorted over the wave range of 4,000 to 500 cm$^{-1.}$

6. Particle size analysis

Particle size of microspheres were determined by optical microscopy .Mean particle size was determined for all formulations of microspheres.

7. Scanning electron microscopy (SEM)

The Surface and cross –section morphologies of the F10 Beads Were observed using a (SEM) (JSM-6490 LA,JEOL,TOKYO,JAPAN) Operated at an acceleration voltage of 25kv.The beads were made conductive by sputtering thin coat of platinum under vacuum using Jeol JFC-1600 Auto Fine Coater And Then Images Were Recorded At Different Magnifications.

8. Buoyancy Test

The obtained beads were studied for buoyancy and floating time using USP Apparatus 2(paddle type).one hundred beads were placed in 900ml of 0.1NHCL (PH-1.2)Containing 0.02% Tween 80 and agitated at 50rpm,temperature was maintained at 37C^0 .

9. *In-vitro* release profile of Calcium Alginate Levofloxacin Floating Microspheres

Release of Levofloxacin from the floating Microspheres were determined by dissolution testing method using. Using U.S.P. Type I Apparatus using 0.1N HCL pH 1.2 at 37 ± 0.5 °C and 100rpm .Concentrations of drug were analyzed by U.V.

10. Drug release kinetics

The drug release kinetics of F10 was studied by various kinetic models as zero order, first order ,Higuchi's model , Koresmeyer Peppas model.

3. Results

1. Preparation of calcium alginate Levofloxacin Floating Microspheres

Microspheres of F1 to F11 were formulated by using different concentrations of Levofloxacin, Sodium Alginate, Chitosan and other excipients as mentioned in Methodology . Microspheres' were dried and stored at room temperature.

Figure 2. Dried Microspheres of F1, F2,F3, F4 , F5, F6, F7, F8,F9,F10 and F11
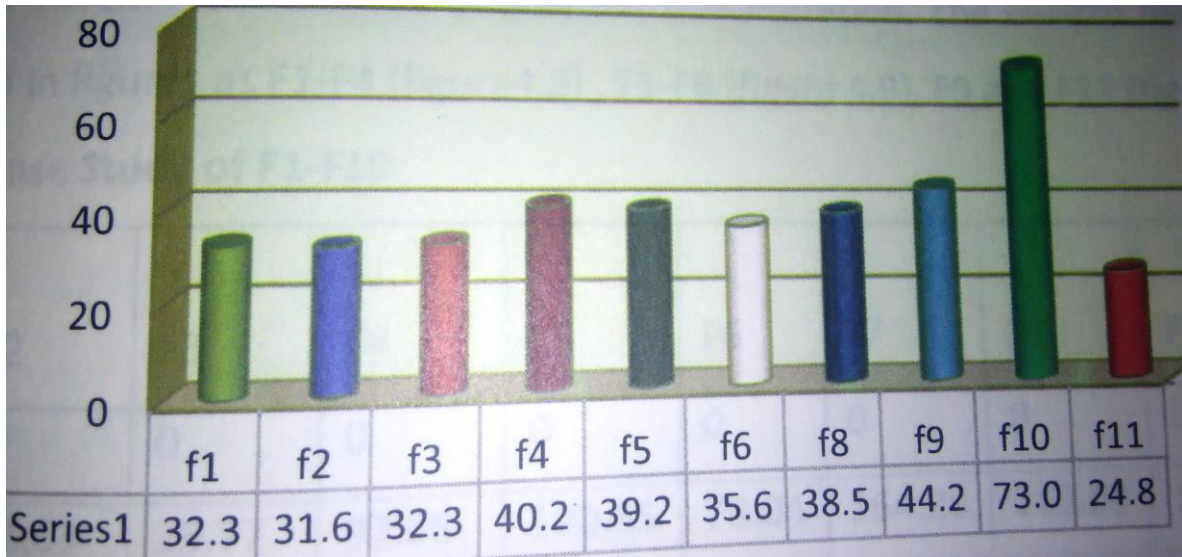
2. Encapsulation Efficiency

Figure 3.Percent Encapsulation Efficiency of Microspheres

The drug encapsulation was increased with increase in drug –polymer ratio for F1the %
Encapsulation was 32.3% with drug polymer ratio of 1:2. With same ratio optimum %
Encapsulation of 73 % was observed in F10. It may be due to Chitosan Complex Present in
F10. F7 Spheres were not In Shape it was not included for characterization.

3. DSC and TGA analysis

The DSC – TGA Thermo gram of selected formulation std. Levofloxacin and F10 shows there were
no interaction between drug and polymers used in F10. Moisture Loss was observed with change
in enthalpy of F10 Higher temperature may be due to other excipients in F10.(FIgure 4 , 5).
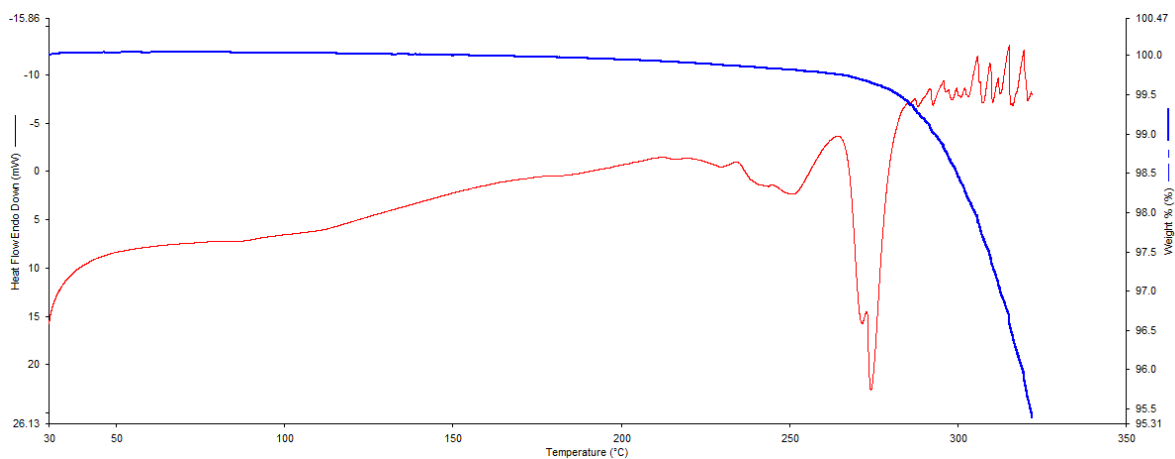
Figure 4.DSC and TGA study of Levofloxacin hemihydrate
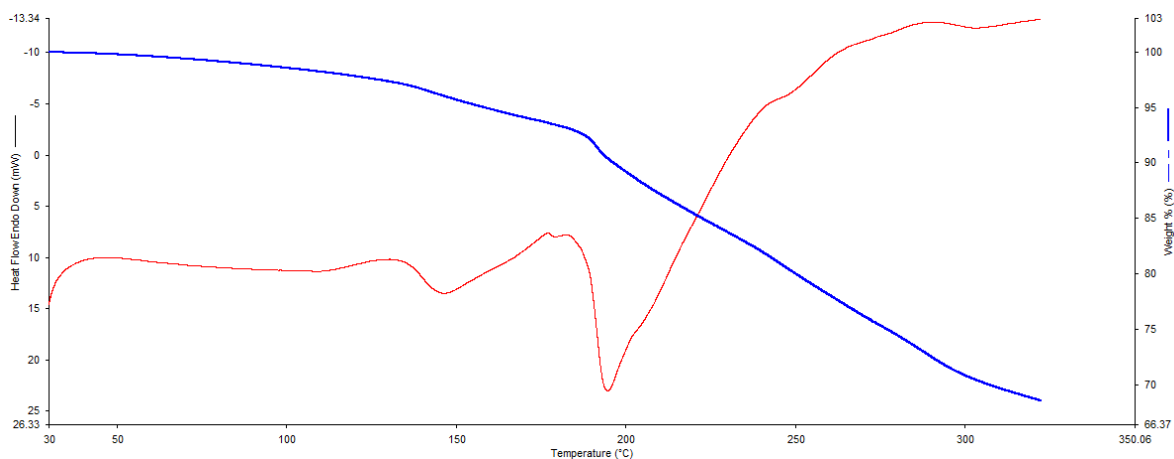
Figure 5. DSC and TGA study of F10

4. FTIR Analysis

FTIR spectra were confirmed the Characteristics peaks compared with FTIR of Std. levofloxacin (Figure 6,7).

Frequency cm^{-1} Standard Levofloxacin	Frequency cm^{-1} Sample of levofloxacin	Possible groups assignment
3427	3427.51	Free Carboxylic-group (COOH). (Stretch)
3100 cm$_{-1}$	3043.67,2935.66,2866.22 2833.3	-C-H stretch of alkenes
1724	1724.72	- vibration of the carbonyl bond (C=O) (stretch)
1600	1622.13,1456.26	C=C Aromatic Group .(streach)

Table 2.0 FTIR Interpretation of Formulation F10.
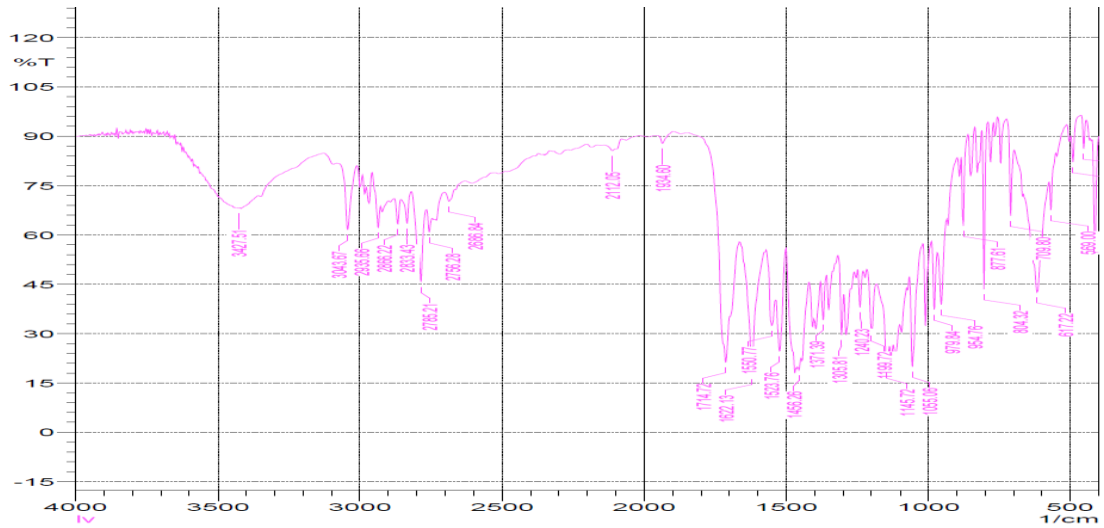
Figure 6. FTIR of Levofloxacin hemihydrate
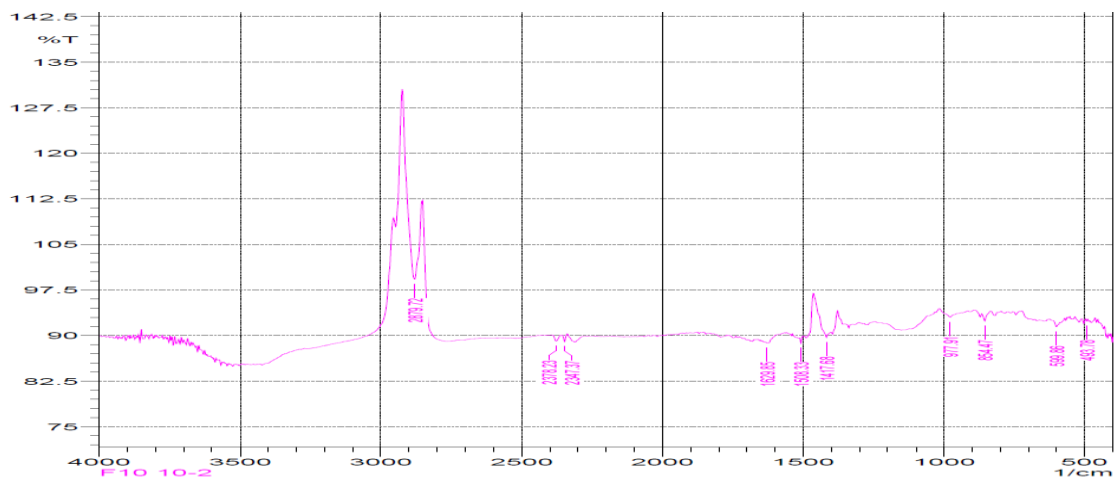
Figure 7.FTIR of F10

5. Particle Size Analysis

The particle size of prepared microspheres was determined by optical microscopy. Mean particle size was in range of 1.43±0.2 to 3.08±0.45 µm. the mean particle size of F10 was 2.24±0.1.

6. Scanning electron microscopy (SEM)

The surface morphology of F10 Microspheres was evaluated by SEM, it was observed that F10 Microspheres were Spherical in shape, with porous and rough surface. (Figure 8) and cross section of F10 shows embedded matrix of Calcium Alginate, Chitosan and levofloxacin (Figure 9).
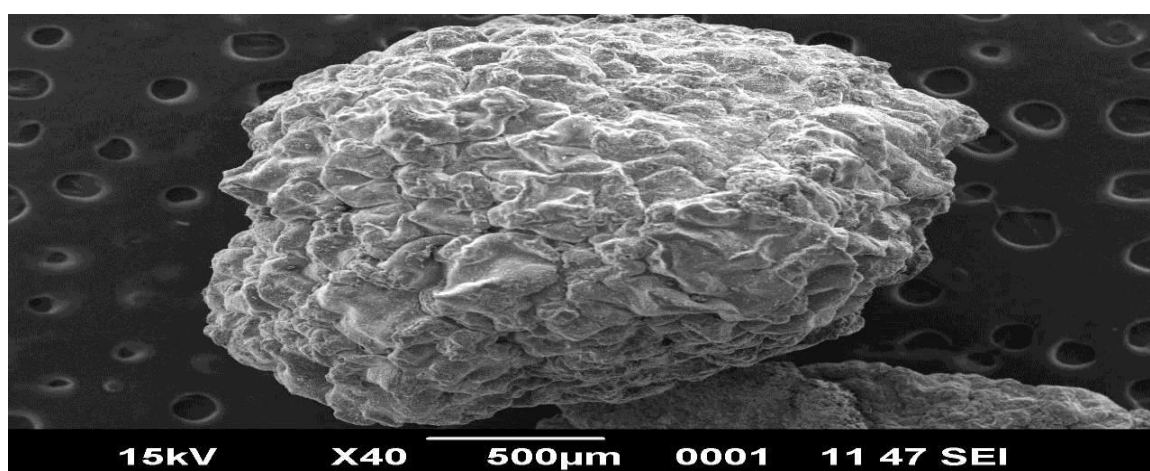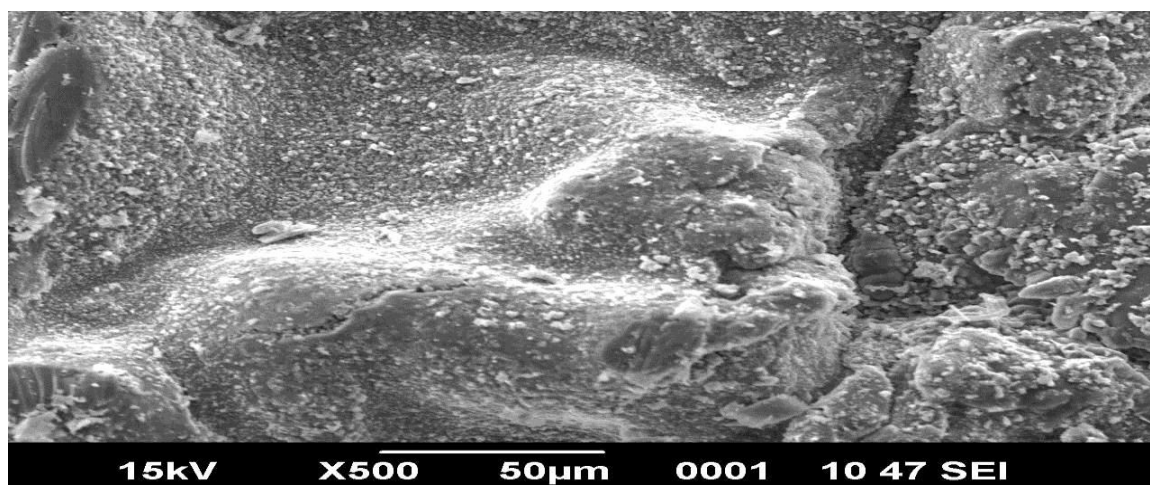

Figure 8.SEM of F10 Microsphere

Figure 9. SEM of Cross Section of F10 Microsphere

7. Buoyancy Test

The F10 Shows 100 % buoyancy, with buoyancy time Raft of 255 sec and F10 shows 12 Hrs of Floating Time , confirmed the floating Behavior of F10.

8. *In-vitro* Drug Release of F10

The F10 showed increased release of drug as compare to other formulations the % cumulative release of F10 at the end of 9 hrs was found to be 134.29% , the % Drug release Vs Time (Hrs)   Represented in Figure 10.
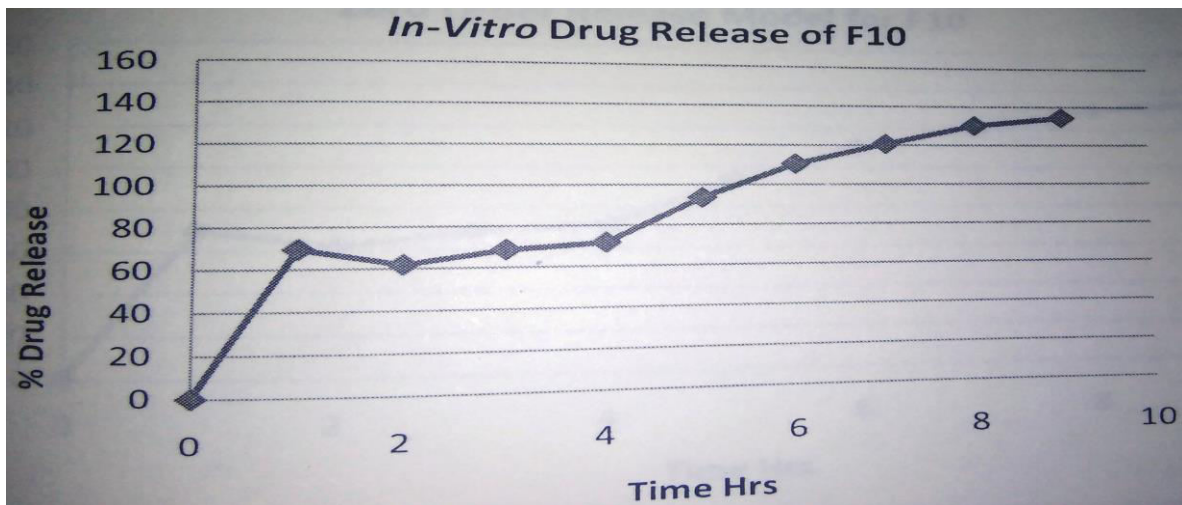

Figure 10.   *In-vitro* drug release from F10 Microspheres

9. Drug release kinetics

The drug release kinetics of F10 was studied by Fitting release data in various kinetic models as zero order, first order ,Higuchi's model , Koresmeyer Peppas model . the regression coefficient (R^2) Values Were Obtained The best linearity was found in Higuchis Equation Plot (R^2= 0.9310) Indicating the release of the drug from matrix as Square Root of time dependent process based on fickian diffusion. The other model data represented in table 3.0 and Figure11.

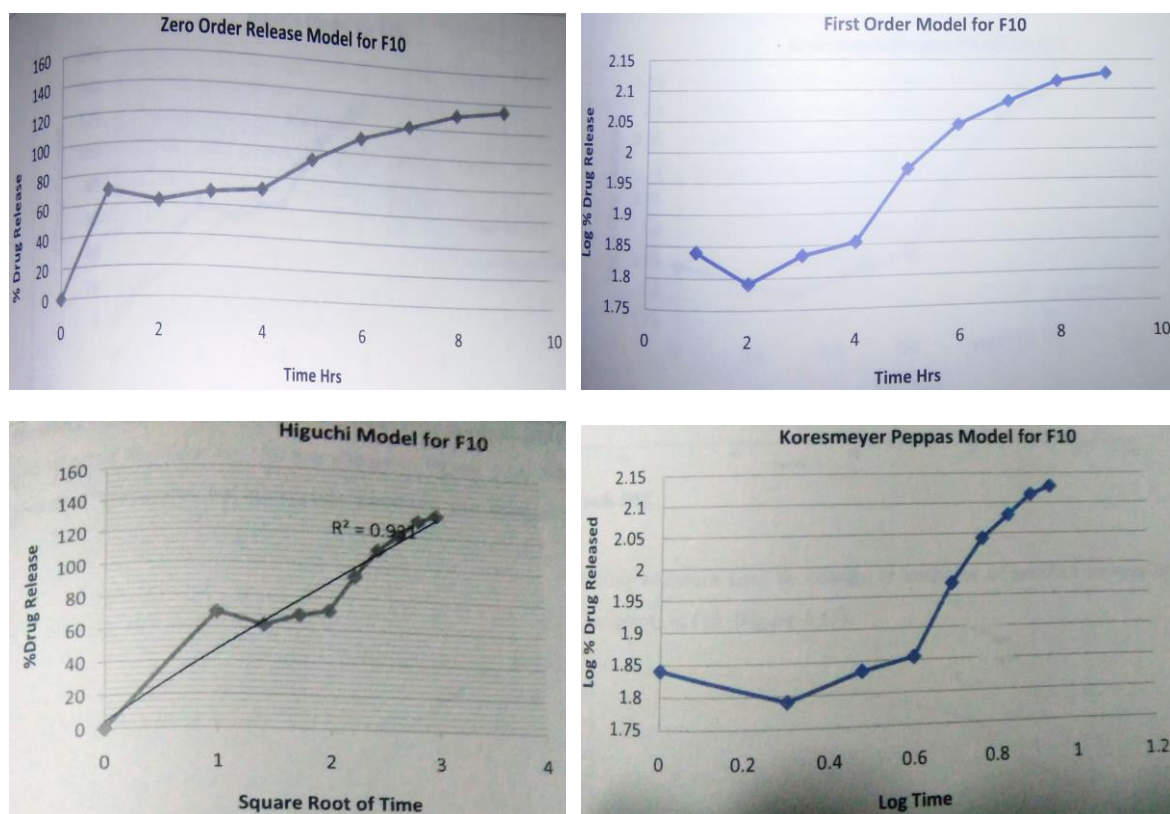| Zero Order	First Order	Higuchi Equation	Koresmeyer Peppas Equation
$Q=K_0 t$	$In(100-Q)=In(Q_0)-K_1 t$	$Q= K_H t^{1/2}$	$Log (Q/100) =k_P t^n$
$R2=0.8759/ K_0 =14.92$	$R2=0.9124/ K_1 =0.0$	$R2=0.9310/ K_H=44.76$	$R2=0.7452/ k_P=0.0142$

Table 3.0 Drug Release Kinetics for F10.

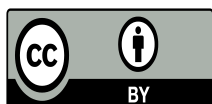**Figure 11. Drug Release Kinetics of F10 Microspheres.**

Conflicts of Interest: "The authors declare no conflict of interest."

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