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Formulation and Evaluation of Floating Microspheres of Amoxicillin Trihydrate

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ABSTRACT

The present study involves the preparation and evaluation of floating microspheres of Amoxicillin Trihydrate for improving the drug bioavailability by prolongation of gastric residence time. Microsphere of Amoxicillin Trihydrate was prepared by emulsion–solvent diffusion method by using Eudragit RS100 and HPMC as a polymer. Eight different formulations were developed naming F1 to F8. The developed floating microspheres were evaluated for percentage yield, particle size, and entrapment efficiency, *in-vitro* buoyancy, scanning electron microscopy and drug release. Surface morphology of formulation F4 exhibited a smooth surface of the floating micro balloons. F4 formulation showed an appropriate balance between buoyancy and drug release rate of 99.12% in 12 hours, which considered the best formulation. Stability study was carried out for the F4 formulation by exposing it to different temperature 5-8°C, 27°C and 40°C for 3 months. The sample was analyzed for drug content at the regular intervals. Instability study, there was no remarkable change in the content of F4 formulation during 30 days in which it was stored at various temperatures. The design system F_4 floats in the stomach and prolongs the gastric residence time (GRT) consequently, providing sustained action. In addition, hollow microspheres enabled increased drug absorption rate, as it gradually sank in the stomach and arrived at the absorption site. The developed formulation overcomes the drawbacks and limitations of sustained-release preparations. Therefore multiple unit floating system, i.e., hollow microsphere will be possibly beneficial for sustained action.

INTRODUCTION

Since the last three decades, many drug molecules formulated as Gastroretentive Drug Delivery System (GRDDS) have been patented keeping in view its commercial success.[1] Oral delivery of drugs is by far the preferable route of drug delivery due to ease of administration, patient compliance, and flexibility in formulation etc. From immediate release to site-specific delivery, oral dosage forms have really progressed. However, it is a well-accepted fact that it is difficult to predict the real *in-vivo* time to release with solid, oral controlled release dosage forms. Thus, drug absorption in the gastrointestinal tract may be very short and highly variable in certain circumstances.[2]

The floating microspheres beneficially alter the absorption of a drug, thus enhancing its bioavailability. They prolong dosing intervals which would allow development of once a day formulations and thereby increase patient compliance beyond the level of existing dosage forms by achieving control over gastric residence time.[3,4]Floating microspheres are gastro-retentive drug delivery systems based on a non-effervescent approach. These microspheres are characteristically free-flowing powders having a size < 199µm and remain buoyant over gastric contents for a prolonged period. As the system floats over gastric contents, the drug is released slowly at the desired rate, resulting in increased gastric retention with reduced fluctuations in plasma drug concentration.[5]

Amoxicillin is an antibiotic useful for the treatment of a number of bacterial infections. It is the first line treatment for middle ear infections.[6] It may also be used for strep throat, pneumonia, skin infections and urinary tract infections among others. It is taken by mouth, or less commonly by injection.[7]

The main aim of the present work was to formulate and evaluate floating microspheres of amoxicillin Trihydrate, which after oral administration could prolong the gastric residence time and increase the drug bioavailability.

MATERIALS AND METHODS:

MATERIALS:

Amoxicillin Trihydrate was obtained from Symbiotic Laboratory ltd as a gift sample. Eudragit RS 100 was obtained from Otto Chemie Pvt Ltd and HPMC from Oxford

laboratory. Dichloromethane, Ethanol, Conc. Hydrochloric acid, Sodium Hydroxide, Tween 20, N-Hexanes, Glyceryl monostearate and Polyvinyl Alcohol were obtained from Vishal Chemicals Mumbai. Disodium Hydrogen Phosphate was obtained from Tirupati Industries, India.

METHODS:

Preparation of Floating Microsphere of Amoxicillin Trihydrate:

Floating microsphere containing Amoxicillin Trihydrate was prepared using emulsion solvent diffusion technique. The drug to polymer ratio used to prepare the different formulations was 1:7.

The polymer content was a mixture of Eudragit RS 100 Hydroxypropylmethylcellulose (HPMC) as shown in Table I. The drug-polymer mixture is dissolved in a mixture of ethanol (8 ml) and dichloromethane (8ml) was dropped into 0.75% polyvinyl alcohol solution (200 ml).

The solution was stirred with a propeller-type agitator at 40°C temperature for 1 hour at 300 rpm. The formed floating microspheres were passed through sieve no.18, 30 and washed with water and dried at room temperature in desiccators. The various batches of floating microsphere were prepared as follows.

EVALUATION OF MICROSPHERES

Particle size analysis:

The Particle size analysis plays an important role in determining the release characteristics and floating property.

The sizes of floating microspheres were measured by using an optical microscope, and the mean particle size was calculated by measuring nearly 200 particles with the help of a calculated ocular micrometer.

Floating behavior of Floating microsphere:

The floating microsphere of 100 mg was weighted and placed in 0.1 N HCI (300 ml) containing 0.02% of tween 20.

The mixture was stirred with a paddle at 100rpm. The layer of buoyant microspheres was pipetted and separated by filtration at 1, 2, 4 and 6 hours.

The collected microspheres were dried in a desiccator overnight. The percentage of microspheres was calculated by the following equation:

$$\%\text{Floating microsheres} = \frac{\text{weight of floating microspheres}}{\text{Initial weight of floating microsheres}} \times 100$$

Drug Entrapment:

The floating microspheres of various formulations were subjected to drug content. 50 mg of floating microspheres from all batches were accurately weighed and crushed.

The powdered of microspheres were dissolved in 10ml ethanol in a 100ml volumetric flask and make up the volume with 0.1 N HCl. This resulting solution is then filtered through Whatmann filter paper.

After the filtration, from this solution, 10 ml was taken out and diluted up to 100 ml with 0.1 N HCl. Again from this solution 2 ml was taken out and diluted up to 10 m1 with 0.1 N HCI and the absorbance was measured at 334.5 nm against 0.1 N HCI as a blank.

The percentage drug entrapment was calculated as follows.

$$\%\text{Drug entrapment} = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} \times 100$$

Flow properties:

The flow properties of the Amoxicillin Trihydrate microspheres were characterized in terms of angle of repose, Hausner ratio and carr index. For determination of an angle of repose (Ɵ), the microspheres were poured through the walls of a funnel, which was fixed at a position such that its lower tip was at a height of exactly 2.0 cm above the hard surface. The microspheres were poured till the time when upper tip of the pile surface touched the lower tip of the funnel. The $\tan^{-1}$ of the height of the pile/ radius of its base gave the angle of repose.

Microspheres were poured gently through a glass funnel into a graduated cylinder cut exactly to 10ml mark. Excess microspheres were removed using a spatula and the weight of the

cylinder with pellets required for filling the cylinder volume was calculated. The cylinder was then tapped from a height of 2.0 cm until the time when there was no more decrease in the volume. Bulk Density (ρB)and Tapped Density (ρT) were calculated. Hausner ratio (H) and carr index (C) were calculated according to the equation given below:

$$H = \rho B/\rho T$$

$$C = 100(1 - \rho B/\rho T)$$

Shape and Surface Characterization of Floating Microspheres by Scanning Electron Microscopy:

From the formulated batches of floating microspheres, formulations (F_4) which showed an appropriate balance between the buoyancy and the percentage release were examined for surface morphology and shape using scanning electron microscope.

The sample was fixed on carbon tape and fine gold sputtering was applied in a high vacuum evaporator. The acceleration voltage was set at 30KV during scanning. Microphotographs were taken on different magnification and higher magnification (500X) was used for surface morphology.

***In-vitro* Release Studies:**

The drug release rate from floating microspheres was carried out using the USP type II (Electro Lab.) dissolution paddle assembly. A weighed amount of floating microspheres equivalent to 100 mg drug were dispersed in 900 ml of 0.1 N HCI (pH 1.2) maintained at 37 ± 0.5°C and stirred at 100 rpm. One ml sample was withdrawn at predetermined intervals and filtered and equal volume of dissolution medium was replaced in the vessel after each withdrawal to maintain sink condition. The collected samples were suitably diluted with 0.1 N HCI and analyzed spectrophotometrically at 334.5 nm to determine the concentration of drug present in the dissolution medium. The dissolution studies were repeated using phosphate buffer pH 6.8 as dissolution medium.

Drug Release Kinetic Data Analysis:

The study the release kinetics of Amoxicillin Trihydrate from the floating microspheres the release data was fitted to these three equations

Zero-order equation: When a graph of the cumulative percentage of the drug released from the matrix against time is plotted, zero order release is linear in such a plot, indicating that the release rate is independent of concentration.

$Q_t = k_0.t$

Where $Q_{t\ is}$ the percentage of drug released at time t and k_0 is the release rate constant;

First order equation: $In\ (100\text{-}Q_t) = In\ 100\text{-}\ k_I.t$

Where k_I is the release rate constant;

Higuchi's equation: $Q_t = k_H.t^{1/2}$

Where K_H is the Higuchi release rate constant

Korseymeyers-Peppas:

The curves plotted may have different slopes, and hence it becomes difficult to exact pin-point which curve follows perfect zero order release kinetics. Therefore, to confirm the kinetics of drug release, data were also analyzed using Korsemeyer's equation.

$$Q_t/Q_\infty = k_{KP}.t^n$$

Where Q_t/ Q_∞ is the fraction of drug released at time t, k_{KP} a constant compromising the structural and geometric characteristics of the device and n is the release exponent.

The slope of the linear curve gives the 'n' value. Peppas stated that the above equation could adequately describe the release of solutes from slabs, spheres, cylinders, and discs, regardless of the release mechanism. The value of 'n' gives an indication of the release mechanism. When n = 1, the release rate is independent of time (typical zero order release / case II transport); n = 0.5 for Fickian release (diffusion/ case I transport); and when 0.5 < n < 1, anomalous (non-Fickian or coupled diffusion/ relaxation) are implicated. Lastly, when n > 1.0 super case II transport is apparent. 'n' is the slope value of log M_t/M_∞ versus log time curve

Stability Study:

From the prepared floating microspheres F_4 which showed an appropriate balance between the buoyancy and the percentage release was selected for stability studies.

The prepared formulation (F_4) were placed in borosilicate screw-capped glass containers and stored at different temperature (27 ± 2°C), oven temperature (40±2°C) and in the refrigerator (5-8°C) for a period of 30 days. The samples were assayed for drug content at regular intervals of two weeks.

RESULT AND DISCUSSION:

EVALUATION OF MICROSPHERES:

Particle size analysis:

Particle size was determined by Optical microscopy method. It plays important role in floating ability and release of drug from microballoon. If a size of microballoons is less than 500 μm release rate of the drug will be high and floating ability will reduce, white micro balloons ranging between 500μm - 1000μm, the floating ability will be more and release rate will be in a sustained manner.The mean particle size of hollow microsphere was in range 509. - 774 μm as shown in Table II.

Floating behavior of microsphere:

Hollow Microsphere was dispersed in 0.1 HCl containing Tween 20 (0.02% w/v) to simulate gastric fluid. Floating ability of different formulation was found to be differed according to Eudragit and HPMC ratio. F_1-F_4 formulations showed best floating ability (91.47-72.97%) in 6 hours. F_5-F_8 formulation showed less floating ability (66.12-36.18%) as showed in Table III. The floating ability of microsphere is decreased by increasing the HPMC ratio.

Drug Entrapment:

The drug entrapment efficacies of different formulations were in a range of 40.31 - 75.18 % w/w as shown in Table IV. Drug entrapment efficacy slightly decreases with increase HPMC content and decreased Eudragit ratio in micro balloons. This is due to the permeation characteristics of HPMC that could facilitate the diffusion of part of entrapped drug to surrounding medium during the preparation of hollow microspheres.

Percentage Yield:

Percentage yield of the different formulation was determined by weighing the micro balloons after drying. The percentage yield of the different formulation was in the range of 53.34 - 81.86% as shown in Table V.

Flow Properties:

The angle of repose, Hausner ratio and carr index were determined to predict flowability. A higher Hausner ratio indicates greater cohesion between particles while a higher carr index is indicative of the tendency to form bridges. The prepared microspheres exhibited good flow properties. The detail result is given in Table V.

Scanning Electronic Microscopy:

Shape and surface characteristic of hollow microspheres examine by Scanning Electronic Microscopy analysis as shown in Figure No. 2, 3. Surface morphology of F_4 formulation examines at to different magnification 40X and 200X, which illustrate the smooth surface of floating micro balloons and small hollow cavity present in microsphere which is responsible for floating property.

***In vitro* Drug release study:**

In vitro drug release study of microballoons was evaluated in 0.1 N HCl and phosphate buffer pH 6.8. Eudragit RS100 which is present in all formulation has a low permeability in acid medium.Since Eudragit is less soluble in acidic pH, a release of drug in 0.1 N HCl was generally low compared to phosphate buffer 6.8 Release rate of F_1, F_2, F_3 formulations (42.791%, 55.311%, and 78.809% respectively). It was found to be slow and incomplete in both dissolution medium. In order to increase the release rate of the drug, the ratio of Eudragit and HPMC is decreased and increased respectively. F_5, F_6, F_7, F_8 (93.681%, 96.348%, 95.295%, 94.329% respectively) formulations showed high release rate with less floating property. F_4 formulation showed a best appropriate balance between buoyancy and drug release rate.

Release Kinetic:

Drug release pattern was evaluated in 0.1 N HCl and phosphate buffer pH 6.8 and the result were given in Table VI and VII. The release rate of F_1, F_2, F_3 formulations was found to be slow and incomplete in both dissolution medium. It was found that drug release rate increased by decreasing and increasing the ratio of Eudragit and the HPMC respectively. Kinetics and mechanism of drug release from all formulation were evaluated on the basis of zero order, Higuchi equation and Peppas model. A correlation coefficient (r^2) and slope value for each equation in the range of (r^2=0.752-0.937) and n=0.568-0.785 were calculated. Zero-order plots for all formulations were found to be linear in acidic and buffer solution of pH 6.8.Which indicates that it may follow zero order kinetics.

Higuchi plot was found to be linear, which indicates diffusion may be the mechanism of drug release for each formulation. Peppas plot was found good linear, n > 0.5 for all formulations, indicated that drug release may follow anomalous diffusion (range=0.993-0.998).

Zero-order plots for F_4 formulation was found to be linear in both dissolution medium, it considered as the best fit for drug release. That indicates it may follow zero-order mechanism. (Refer Figure No.4, 5,6,7,8 and 9).

Stability Study:

Stability study was carried out for the F4 formulation by exposing it to different temperature 5-8°C, 27°C and 40°C for 3 months. The sample was analyzed for drug content at the regular intervals. It was found that no remarkable change in the drug content of the F_4 formulation. This indicates that F_4 was stable for the following temperature. The stability study data was given in Table VIII.

SUMMARY AND CONCLUSION:

Microsphere of Amoxicillin Trihydrate was prepared by emulsion–solvent diffusion method by using Eudragit RS100 and HPMC as a polymer. Mean particle size range for all formulation was varied from 509 to 774 μm, due to change in drug and polymer ratio. Drug entrapment of all formulation was found in a range of 41.32 to 76.19% w/w and its efficiency slightly decreases with increasing the HPMC content. Ideal property of microspheres includes high buoyancy and sufficient release of drug in pH 6.8. F_4 formulation showed the

appropriate balance between buoyancy and drug release rate of 99.12% in 12 hours, which is considered as the best formulation.

In vitro data obtained from floating microspheres of Amoxicillin trihydrate showed excellent floatability, good buoyancy, and prolonged drug release. The design system F_4 floats in the stomach and prolongs the gastric residence time (GRT) consequently, providing sustained action. In addition, hollow microspheres enabled increased drug absorption rate, as it gradually sank in the stomach and arrived at the absorption site. The developed formulation overcomes the drawbacks and limitations of sustained-release preparations. Therefore multiple unit floating system, i.e., hollow microsphere will be possibly beneficial for sustained action. Thus the aim of the study to formulate floating microspheres of Amoxicillin Trihydrate was achieved. In future, this system can be developed by using various polymers in various proportions for better results.

Table 1: Formulation of the Floating Microspheres prepared

Sr. No	Formulation Code	Amoxicillin Trihydrate (gm)	Eudragit Rs 100 (gm)	HPMC (gm)
1	F_1	0.1	0.7	0.0
2	F_2	0.1	0.6	0.1
3	F_3	0.1	0.5	0.2
4	F_4	0.1	0.4	0.3
5	F_5	0.1	0.3	0.4
6	F_6	0.1	0.2	0.5
7	F_7	0.1	0.1	0.6
8	F_8	0.1	0.0	0.7

***HPMC = Hydroxypropylmethylcellulose**

Table 2: Mean particle size of Different Batches of the microsphere

Sr. No	Formulation code	Mean particle size(μm)
1	F_1	774
2	F_2	736
3	F_3	694
4	F_4	676
5	F_5	652
6	F_6	648
7	F_7	532
8	F_8	509

Table 3: Percentage Buoyancy for Different Formulation

Formulation	1 hour	2 hours	4 hours	6 hours
F_1	97.40	96.07	92.22	90.46
F_2	97.10	94.57	91.16	86.33
F_3	97.53	94.63	84.33	77.44
F_4	98.53	91.48	79.56	71.96
F_5	97.71	90.94	72.40	65.11
F_6	97.44	85.61	64.13	56.75
F_7	88.34	75.41	56.04	45.09
F_8	80.50	66.22	51.19	35.17

Table 4: Drug Entrapment for Different Formulation

Formulation	Drug entrapment(% w/w)
F_1	75.18
F_2	69.58
F_3	65.22
F_4	63.45
F_5	60.01
F_6	58.37
F_7	47.45
F_8	40.31

Table 5: Percentage Yield, True Density, Tapped Density, % Compressibility Index and Angle of Repose of Different Formulations

Formulation	Percent Yield (%)	True Density (gm/cm^3)	Tapped Density (gm/cm3)	% Compressibility Index	Angle of Repose
F_1	81.86	0.375	0.132	7.39	24°.39’
F_2	77.52	0.418	0.156	8.77	26°.82’
F_3	75.46	0.437	0.167	8.46	28°.68’
F_4	70.55	0.589	0.179	10.63	28°.18’
F_5	68.30	0.597	0.231	12.49	30°.39’
F_6	65.02	0.616	0.264	11.67	32°.81’
F_7	55.83	0.753	0.275	15.45	34°.54’
F_8	53.34	0.875	0.315	16.68	36°.72’

Table 6: Release Kinetics of Microsphere in 0.1 N HCl

Formulation	Zero Order		Higuchi Equation		Peppas Equation	
	r^2	K_0	r^2	K_H	r^2	N
F_1	0.950	1.81	0.989	6.946	0.937	0.756
F_2	0.954	2.08	0.998	8.141	0.817	0.785
F_3	0.963	2.86	0.994	11.04	0.872	0.769
F_4	0.948	3.49	0.996	13.66	0.835	0.634
F_5	0.930	4.03	0.993	16.09	0.752	0.664
F_6	0.964	4.68	0.996	18.08	0.822	0.612
F_7	0.956	5.80	0.998	22.42	0.833	0.581
F_8	0.954	5.85	0.997	22.86	0.759	0.568

*r^2 =Correlation coefficient, K_0= release rate constant, K_H=Higuchi release rate constant, N=release exponent

Table 7: Release Kinetics of Microsphere in Phosphate Buffer PH 6.8

Formulation	Zero Order		Higuchi Equation		Peppas Equation	
	r^2	K_0	r^2	K_H	r^2	N
F_1	0.997	3.761	0.978	13.73	0.920	0.756
F_2	0.982	5.92	0.973	21.84	0.937	0.785
F_3	0.984	7.65	0.965	27.69	0.941	0.769
F_4	0.991	8.29	0.982	30.54	0.890	0.634
F_5	0.969	8.84	0.987	33.49	0.843	0.664
F_6	0.950	8.67	0.988	33.04	0.794	0.612
F_7	0.955	8.31	0.992	32.43	0.784	0.581
F_8	0.937	8.44	0.985	33.02	0.771	0.568

*r^2 =Correlation coefficient, K_0= release rate constant, K_H=Higuchi release rate constant, N=release exponent

Table 8: Stability Study Data for F4 Formulation

S. No	Days	% Drug Remaining 5-8°C	% Drug Remaining 27±2°C	% Drug Remaining 42±2°C
1	0	100 ± 00	100 ± 00	100 ± 00
2	30	99.6 ± 0.015	99.9 ± 0.003	99.4 ± 0.041
3	45	99.5 ± 0.013	99.8 ± 0.027	99.2 ± 0.036
4	90	99.4 ± 0.15	99.6 ± 0.012	99.1 ± 0.02

*** Values are mean ± S.D.**

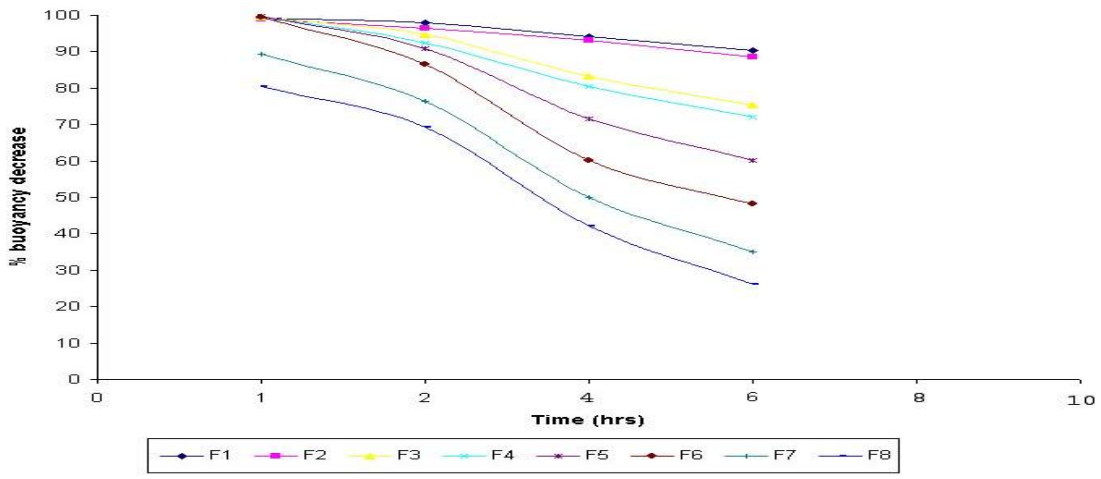

Figure 1: Percent Buoyancy Decreased For Different Formulation

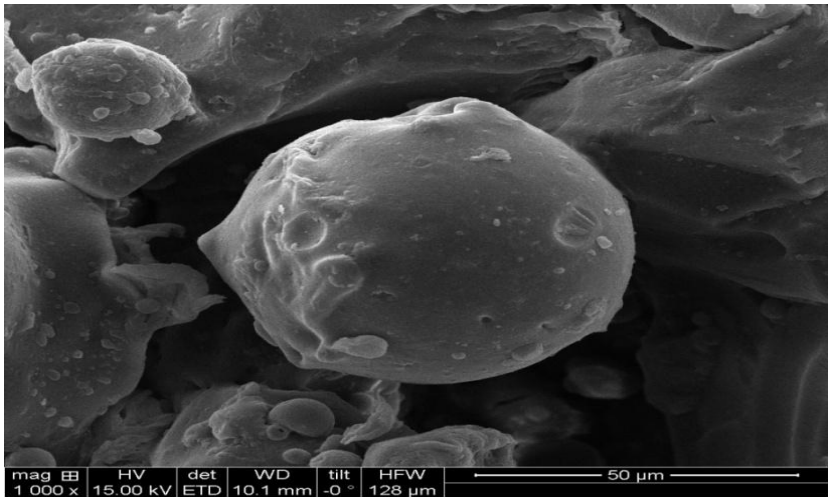

Figure 2: SEM Pictures of prepared best formulation microsphere

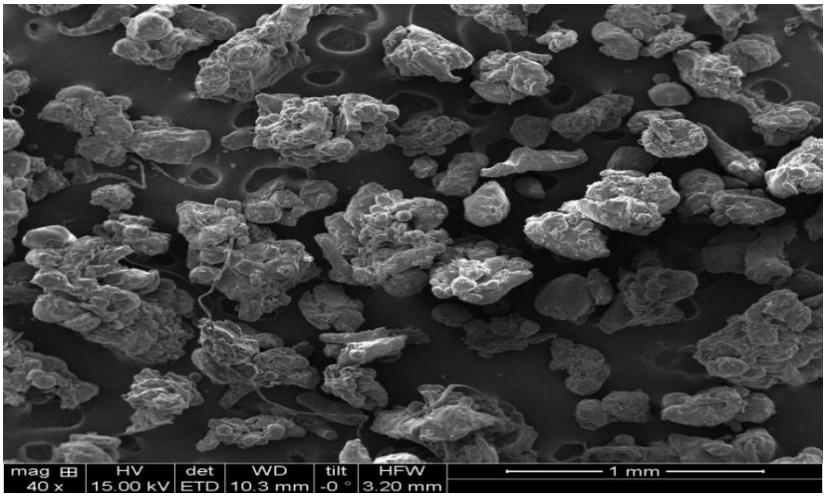

Figure 3: SEM Pictures of prepared best formulation microsphere internal structure

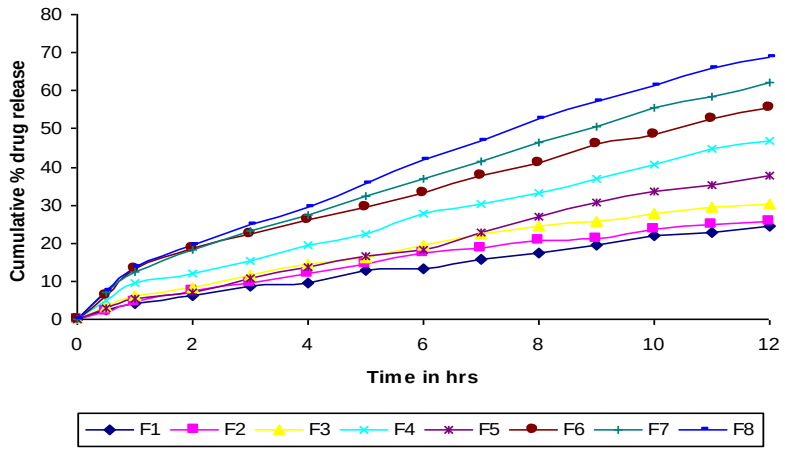

Figure 4: Zero Order Plot for all Formulation in 0.1 N HCl

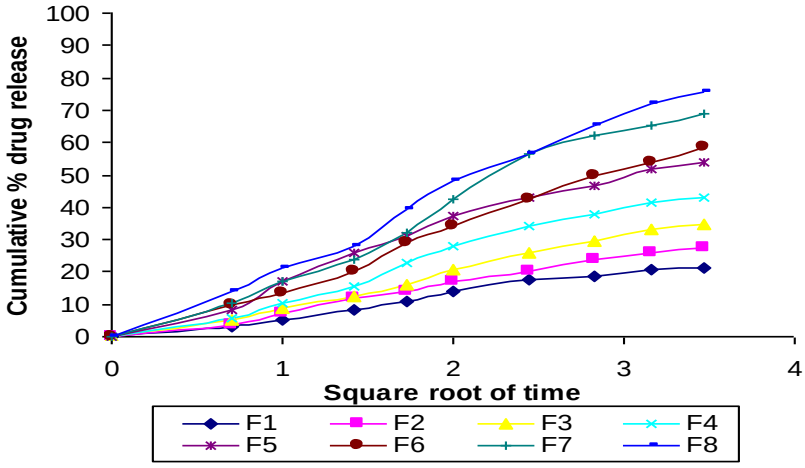

Figure 5: Higuchi Plot for All Formulation in 0.1 N HCl

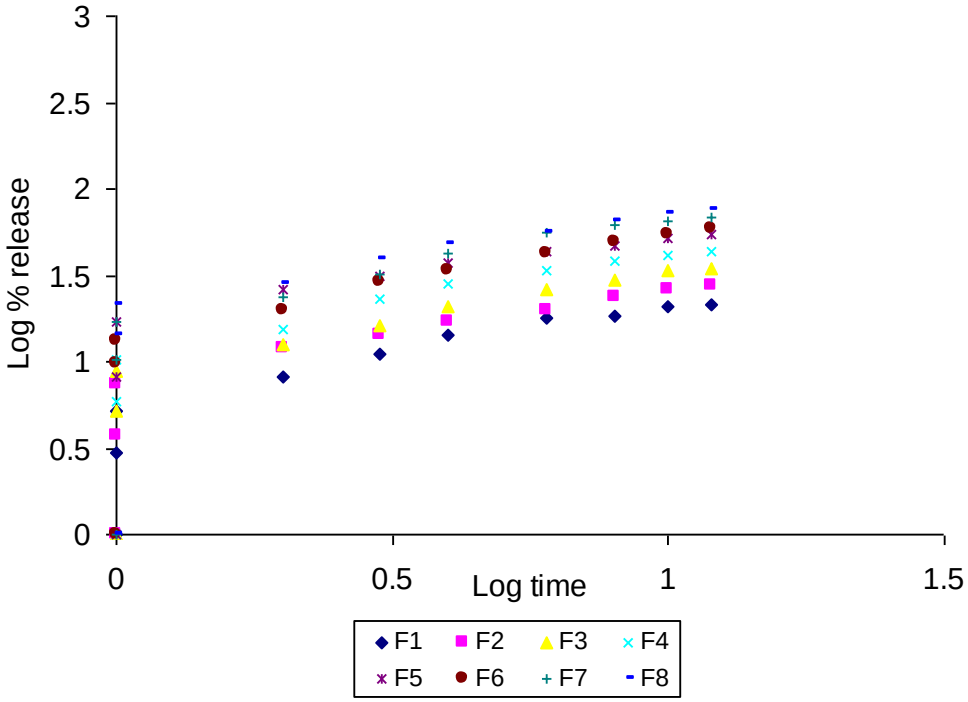

Figure 6: Peppas Plot for All Formulation in 0.1 N HCl

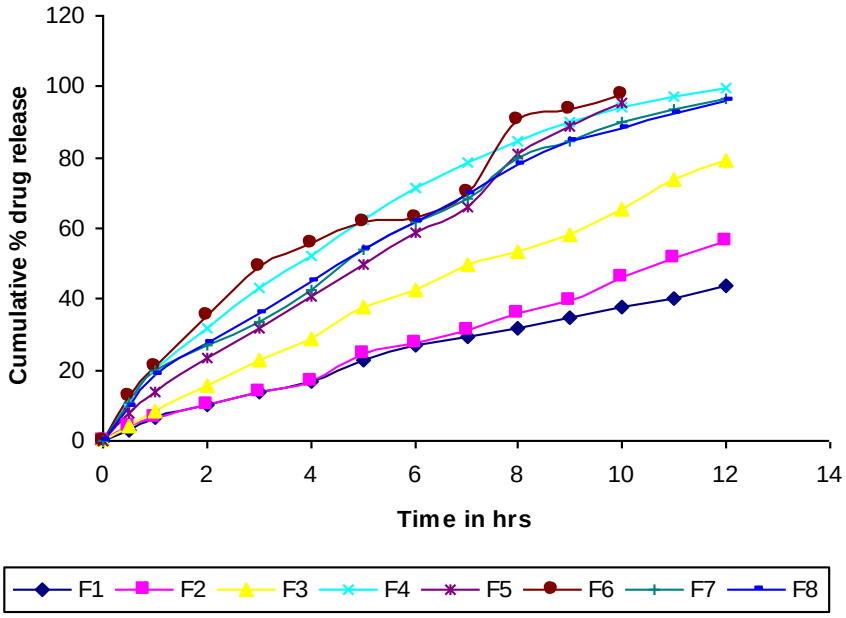

Figure 7: Zero Order Plot for All Formulation in Phosphate Buffer pH 6.8

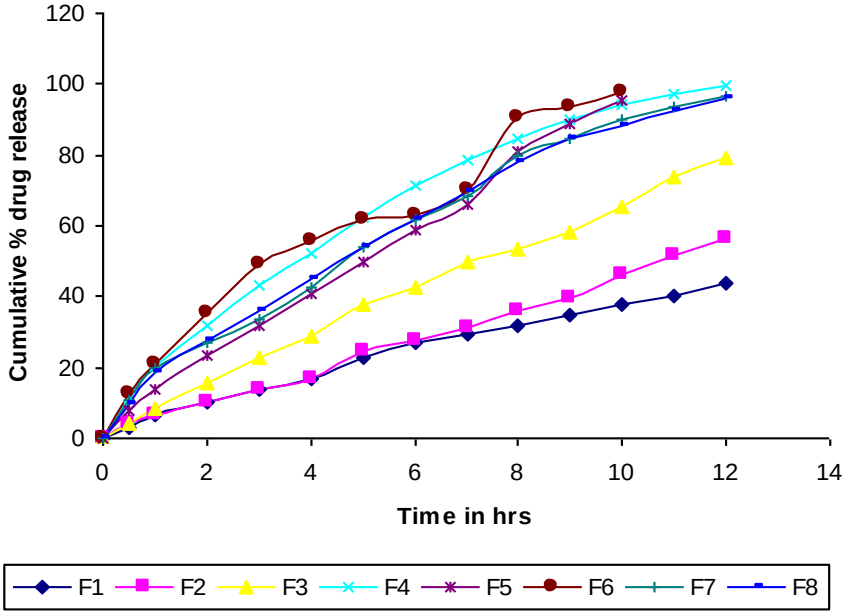

Figure 8: Higuchi Plot for All Formulation in Phosphate Buffer pH 6.8

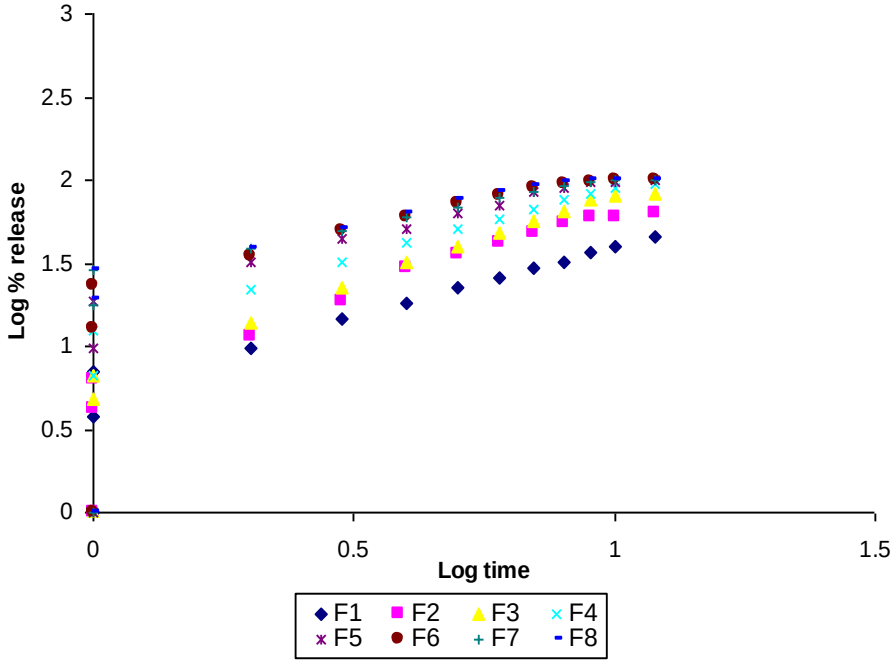

Figure 9: Peppas Plot for All Formulation in Phosphate Buffer pH 6.8

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