DESIGN AND EVALUATION OF FLOATING MICROSPHERES OF AMOXICILLIN TRIHYDRATE BY IONOTROPIC GELATION METHOD

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The purpose of this investigation was to design and develop floating microspheres of Amoxicillin Trihydrate by ionotropic gelation method with combination of two polymers and to get the best possible formulation out of that with the various aspects. Floating drug delivery system have a bulk density less than gastric fluids and so remains buoyant in the stomach without affecting gastric emptying rate for a prolonged period of time. The floating microspheres were prepared using Ethyl cellulose and Hydroxy propylmethyl cellulose K4M as polymer to achieve an extended retention in upper GIT and there by improved bioavailability. The microspheres were evaluated for particle size analysis, Drug Entrapment Efficiency, Drug Loading Capacity, Floating efficiency, Swelling Study, Loose Surface Crystal Study , drug entrapment efficiency, drug-polymer compatibility study, Micromeritic properties like Bulk Density, Tapped Density, Carr's Index, and Hausner's Ratio, *In-vitro* release studies and surface morphology characterized by Scanning electron microscopy (SEM). The Microspheres have an average size range of 743.00±7.000 to 837.00±8.544µm. The entrapment efficiency was found to be in the range of 66.96±1.944 to 82.03±0.657 %. The *In-vitro* release studies of the drug from the best formulation F6 exhibited a sustained release of 93.46±0.684 % as studied over 10hrs. Release was best explained by zero-order kinetics model and it shows that the drug release follows diffusion mechanism. FT-IR data revealed that, compatible and there was no interaction between the drug and excipients added in the formulation. The data obtained in this study thus suggest that a floating microspheres of Amoxicillin Trihydrate are promising for sustained drug delivery which can reduce dosing frequency.

Key words: Microspheres, Amoxicillin Trihydrate, Ionotropic Gelation Method, Ethyl cellulose, Hydroxy propylmethyl cellulose

INTRODUCTION

One of the most feasible approaches for achieving a prolonged and predictable drug delivery in the GI tract is to control the gastric residence time by using gastro-retentive dosage forms (GRDF s). It remains in the gastric region for several hours and hence prolongs the gastric residence time of drug. It has several advantages over immediate release dosage form including the minimization of fluctuations in drug concentration in plasma and at the site of action over prolonged periods of time, resulting in optimized therapeutic efficiencies and reduce the side effect, reduction of total dose administered and reduction of administration frequency leading to improved patient compliances [1- 2].

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Oral controlled release (CR) dosage forms have been developed for the past 3 decades due to their considerable therapeutic advantages. However, this approach has not been suitable for a variety of important drugs, characterized by a narrow absorption window in the upper part of the gastrointestinal tract i.e. stomach and small intestine due to short transit time, resulting lesser bioavailability. Many orally-administered drugs display poor bioavailability (30% or less) when administered in conventional dosage form, i.e., the rate and extent to which the drugs are absorbed is less than desirable indicating requirement of a very large dose. Unabsorbed drug may also have undesirable side effect within the gastrointestinal tract. This problem maybe overcome by modified release drug delivery system with prolonged residence time in the stomach. Systems that prolong the gastric residence time can also be used

as sustained release devices with a reduced frequency of administration by densitycontrolled delivery systems that either float or sink in gastric fluids [3]. Floating microspheres are gastro-retentive drug delivery systems based on non-effervescent approach. These microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers. Solid biodegradable microspheres incorporating a drug dispersed or dissolved throughout particle matrix have the potential for controlled release of drugs. Floating microspheres have emerged as an efficient means of enhancing the bioavailability and controlled delivery of many drugs the increasing sophistication of delivery technology will ensure the development of increasing number of gastro-retentive drug delivery systems to optimize the delivery of molecules that exhibit absorption window, low bioavailability, and extensive first pass metabolism [4, 5]. Amoxicillin is penicillinase-susceptible penicillin is closely related to ampicillin. Amoxicillin inhibit bacterial growth by interfering with a specific step in bacterial cell wall synthesis. The drug is absorbed more rapidly and completely from the GI tract.

MATERIAL AND METHODS

Amoxicillin Trihydrate was obtained as a gift sample from Ranbaxy Laboratory Ltd. Dewas Plant, M.P. Hydroxy propylmethyl cellulose K4M was obtained from Dow Chemical Company, Middlexes, US. Carbopol 940 and sodium alginate were purchased from LobaChemie Pvt Ltd, Bombay. Calcium Chloride was obtained from Merck Specialties Pvt Ltd Bombay.

Preparation of Floating Microspheres:

9 different batches of the Amoxicillin floating microspheres were prepared with different ratio of HPMC K4M, Carbopol 934 and sodium alginate with Calcium chloride as counter ion. 2 gm. Sodium alginate was dissolved in 50 ml distilled water in a beaker by using a mechanical stirrer (Remi Motors, Model No. RO-123R, Mumbai, India.). Then 500mg Amoxicillin and the different amount of HPMC K4M &Carbopol 940 were added consecutively to the alginate solution. Mixed them thoroughly for 1hr with the magnetic stirrer (RemiEquipments, Mumbai, India, model 2MIH). For microsphere formation, the drug-polymer solution was dropped through 21G needle into 100 ml 5%w/v aqueous solution of Calcium chloride. The solution was continuously stirred at 100-rpm by using a magnetic stirrer. After addition of last drop in the calcium chloride solution, it was harvested at room temperature for 10 min. The resultant microspheres were filtered by using Whatman filter paper. The filtrate microspheres were collected in a petridish and washed 2 times with distilled water. Then the formulated microspheres were dried at room temperature for 24 hrs. All batches were prepared in triplicate Details about The floating microspheres formulations are given in the Table 1.

Evaluation of microspheres

***Drug Entrapment Efficiency*[6]:**

The Drug Entrapment Efficiency (DEE) of Amoxicillin floating microspheres was performed by taking the equivalent amount of microspheres in which 10 mg. drug was present. Then the microspheres were crushed and suspended in 10 ml of stimulated gastric fluid 0.1 N HCL (pH 1.2) and kept for 24 hrs. at room temperature. Next day it was stirred for 5 minutes and filtered. 0.25 ml of the filtrate solution was taken in a 10 ml volumetric flask the volume was adjusted with 0.1 N HCL. Then the absorbance was measured spectrophotometrically at 273 nm against pH 1.2 buffer solution as blank using UV- Visible Spectrophotometer (Shimadzu – 1700, Japan).

The absorbance found from UV- Visible Spectrophotometer was plotted on the standard curve to get the concentration of the entrapped drug.

$$DEE = \frac{Practical\ drug\ content}{theoretical\ drug\ content} \times 100$$

All methods of this experiment were performed in triplicate manner for each batch and the average value is taken.

TABLE 1: Lists of Compositions for Nine Batches of the Formulations.

Formulation Code	Drug: Polymer	Drug (mg)	HPMC K4M (mg)	Carbopol 934 (mg)	Sodium alginate (g)	Calcium chloride (g)
F1	1: 6.2	500	450	650	2	5
F2	1:6.4	500	450	750	2	5
F3	1:6.5	500	450	800	2	5
F4	1:6.3	500	500	650	2	5
F5	1:6.5	500	500	750	2	5
F6	1: 6.6	500	500	800	2	5
F7	1:6.6	500	650	650	2	5
F8	1:6.8	500	650	750	2	5
F9	1:6.9	500	650	800	2	5

Drug Loading Capacity [7]**:**

Drug loading Capacity of the formulated floating microsphere was determined by the following formula:

$$Drug\ loading(\%) = \frac{Q_m}{W_m} \times 100$$

Where, W_m = Weight of the microspheres of a batch

Q_m = Quantity of the drug present in Wm microspheres.

All methods of this experiment were performed in triplicate manner for each batch and the average value is taken.

Percentage of Yield:

The Percentage of Yieldfor the microspheres of Amoxicillin is calculated by the following formula:

$$\%Yield = \frac{Practial\ weight\ of\ microsphere}{Expected\ weight\ of\ microsphere} \times 100$$

All methods of this experiment were performed in triplicate manner for each batch and the average value is taken.

Angle of Repose:

Angle of repose of different formulations was measured by taking 3 gm formulated microspheres according to fixed funnel standing method. The microsphere was allowed to flow freely through an orifice from a certain height on a horizontal plane and a conical heap formed on the surface of the plane. The angle which heap formed with the horizontal surface is the Angle of repose that was determined by following formula:

$$angle\ of\ repose(\theta) = \tan^{-1} {}^{h}/_{r}$$

Where, r = the radius of the base of the heap of microsphere; h = height of the heap of microsphere

All methods of this experiment were performed in triplicate manner for each batch and the average value is taken.

Bulk Density & Tapped Density [8-9]**:**

The bulk density and tapped density of the Amoxicillin floating microspheres were measured by using 10 ml of graduated cylinder with 2gm of formulated microspheres. The sample poured in cylinder was tapped mechanically for 100 times, and then tapped volume was noted down. Bulk density and tapped density were calculated.

$$bulk\ density = \frac{weight\ of\ the\ microspheres}{bulk\ volume}$$

$$tapped\ density = \frac{weight\ of\ the\ microspheres}{volume\ after\ tapping}$$

All methods of this experiment were performed in triplicate manner for each batch and the average value is taken.

Carr's Index [8-9]**:**

Compressibility index (Ci) or Carr's Index (CI) value of microparticles was computed according to the following equation:

$$CI = \frac{tapped\ density - bulk\ density}{tapped\ density} \times 100$$

All methods of this experiment were performed in triplicate manner for each batch and the average value is taken.

Hausner Ratio:

Hausner ratio of microparticles was determined by comparing the tapped density to the bulk density using the equation:

$$Hausner\ Ratio = \frac{tapped\ density}{bulk\ density}$$

All methods of this experiment were performed in triplicate manner for each batch and the average value is taken.

Sieve Analysis:

500mg of prepared microspheres were separated into different size fraction by sieving for 10 minutes using a mechanical shaker containing standard sieves having mesh size of #16, #18, and #25. The particle size distribution of the microspheres for all the batches of formulations was determined and mean particle size of microspheres was calculated by using the following formula.

$$mean\ Particle\ size = \frac{\sum(P_f \times W_f)}{\sum W_f}$$

P_f = mean particle size of the fraction

W_f = weight fraction

Surface Topography [8]**:**

The samples for the scanning electron microscopy analysis were prepared by sprinkling the microspheres on one side of an adhesive stub. The microspheres ware then coated with gold in ion sputtering unit and finally the microspheres were mounted into the scanning electron microscope (FEI Quanta-200 MK2, Netherlands).

Compatibility Studies**:**

The compatibility of drug and polymer under the experimental condition is an important prerequisite for the formulation. It is therefore necessary to confirm the drug does not react with the polymer under the experimental condition. It was confirmed by IR Spectral and DSC Thermogram analysis.

Fourier transformed infrared spectroscopy [10]**:**

IR spectroscopy of Amoxicillin Trihydrate loaded Hydroxy propylmethyl Cellulose/ Carbopol Floating microspheres ware performed on fourier transformed infrared spectrophotometer (Perkin Elmer, Spectrum-100, UK). The microspheres of drug and potassium bromide were prepared by compressing the powders at 20 psi for 10 min on KBr press. The mixture was ground into a fine powder using an agate mortar before compressing into a disc and the spectra were scanned in the wave number range of 2000-500 cm-1.8 coupled to a personal computer. The characteristic peaks of IR transmission spectra of Amoxicillin Trihydrate pure drug and Amoxicillin Trihydrate formulation were recorded.

Differential scanning calorimetry (DSC)**:**

Differential scanning calorimetry (DSC) is valuable in studying the beginning of melting of a compound. The temperature at which the suspected melting endothermic peak begins is considered to be the beginning of melting. Thermograms of Amoxicillin Trihydrate - Hydroxy propylmethyl Cellulose/ Carbopol Floating microspheres were obtained using a Perkin Elmer-Jeda DSC instrument equipped with an intra-cooler. Powder samples were hermetically sealed in perforated aluminium pans and heated at a constant rate. Purge gas- Nitrogen at a flow rate 20mi/min and heating temperature of 100 ^{o}C was used to maintain inert atmosphere. (For the DSC thermogram of Amoxicillin, sample consisted of a single sharp endotherm maximum at 158.57°C .The endotherm is assign to the melting of the compound and characterised by an enthalpy of

fusion = 377.4428 J/g .The quality of the thermogram indicates the purity of Amoxicillin.

Floating Efficiency or Percentage of buoyancy [11]**:**

To determine the in-vitro buoyancy, 50 mg formulated Amoxicillin floating microspheres were placed in simulated gastric fluid (pH 1.2, 100 ml) containing 0.02 w/v% Tween 20. The medium was stirred at 100 rpm in a magnetic stirrer (RemiEquipments, Mumbai, India, model 2MIH) and the temperature controlled at 37ºC ± 0.5ºC.

After 8 h, the layer of floating microspheres was pipetted and separated by filtration. The sinking microspheres layers were separated by filtration. Then the both types of microspheres were dried at room temperature. Both the fractions of microspheres were weighed and percentage of buoyancy was determined by the weight ratio of floating particles to the sum of floating and sinking particles.

$$Floating\ efficiency(\%) = \frac{W_f}{W_f + W_s} \times 100$$

All methods of this experiment were performed in triplicate manner for each batch and the average value is taken.

Swelling Studies of Microspheres [12]**:**

For estimating the swelling index, 100mg of microspheres were placed in a beaker with stimulated gastric fluid (pH 1.2) and allow to swell at the temperature 37ºC ± 0.5ºC for required time period.

After 8 hrsThe microspheres were removed and blotted with filter paper. Then their changes in weight were measured. The degree of swelling was then calculated from the following formula:

$$Swelling\ Index = \frac{W_g - W_0}{W_0}$$

Where, W_0= Initial weight of the microspheres, W_g=Weight of the microspheres after swelling

All methods of this experiment were performed in triplicate manner for each batch and the average value is taken.

***Loose Surface Crystal Study (LSC)*:**

To determine the drug adhere loosely on the surface of the microspheres, the formulated microspheres of equivalent to 10 mg of pure drug was suspended in a 10 ml volumetric flask containing stimulated gastric fluid (pH 1.2) and was shaken vigorously for 5 minutes. The drug leached out from the surface of the microspheres into the pH 1.2. 0.25 ml of the drug containing fluid was taken into another 10 ml volumetric flask and volume was adjusted with the buffer solution pH 1.2. The absorbance was measured spectrophotometrically at 273 nm against pH 1.2 buffer solution as blank using UV-Visible Spectrophotometer (Shimadzu – 1700, Japan).

The absorbance was plotted on the standard curve to get the concentration of the entrapped drug.

$$LSC(\%) = \frac{Drug\ leached\ from\ microsphere}{Practial\ drug\ content} \times 100$$

All methods of this experiment were performed in triplicate manner for each batch and the average value is taken.

In-vitro drug release study [13]**:**

In – vitro release rate studies were carried out using dissolution apparatus Type II (USP -XXIV dissolution apparatus, Testing instruments, Kolkata). Equally weighed amount of microsphere containing 250 mg was poured into a tea bag (as a semi permeable membrane) and tied with a paddle. Simulated gastric fluid without enzymes of pH 1.2 was used as dissolution medium (900 ml) and was maintained at temperature 37°C ± 0.5° C. The paddle speed was controlled at 100 rpm. 5 ml of dissolution fluid was withdrawn at regular time intervals up to 10 h and a 5 ml of fresh dissolution fluid was added to replace the sample that was withdrawn. The samples were filtered and accurately diluted. Drug content of the beads was determined by UV/Visible spectroscopy at 273nm.The percentage of drug release from the microsphere was calculated.

Dissolution studies were performed three times for each batch and the mean values are taken.

Table 2: Evaluations of various parameters

Formulation Code	DEE (%)*	Drug Loading (%)*	Yield (%)*	Angle of Repose*	Bulk Density*	Tapped Density*
F1	66.96±1.944	15.58±0.412	89.15±2.31	11.65±0.092	0.810±0.007	0.858±0.003
F2	69.50±0.883	14.89±0.085	90.70±0.50	13.23±0.086	0.705±0.004	0.803±0.008
F3	72.84±1.725	14.67±0.036	90.84±0.20	12.16±0.056	0.721±0.005	0.788±0.004
F4	82.03±0.657	14.86±0.055	92.12±0.34	10.21±0.040	0.683±0.009	0.744±0.012
F5	80.60±1.138	14.63±0.031	91.06±0.19	11.10±0.070	0.723±0.007	0.754±0.005
F6	77.30±1.944	14.84±0.237	88.64±1.40	11.73±0.108	0.707±0.014	0.765±0.012
F7	75.29±1.944	14.63±0.066	89.89±0.41	10.39±0.059	0.685±0.004	0.726±0.004
F8	71.98±1.720	14.25±0.086	89.91±0.55	12.09±0.060	0.700±0.013	0.752±0.008
F9	68.68±1.087	14.20±0.040	89.14±0.26	13.14±0.070	0.723±0.005	0.792±0.004

***mean ± SD (n = 3)**

Formulation Code	Carr's Index*	Hausner Ratio*	Particle size (µm)*	Floating Efficiency (%)*	LSC (%)*	Swelling Index *
F1	5.67±0.48	1.060±0.005	781.00±7.550	60.77±0.71	4.39±0.085	0.787±0.025
F2	12.16±0.36	1.138±0.005	784.00±7.211	62.63±0.58	3.90±0.089	0.817±0.035
F3	8.50±0.27	1.093±0.003	791.67±7.638	65.55±0.98	3.99±0.089	0.907±0.040
F4	8.19±0.41	1.089±0.005	743.00±7.000	87.92±0.71	2.60±0.067	1.313±0.042
F5	5.60±0.35	1.042±0.004	759.00±6.557	84.20±0.18	2.64±0.151	1.257±0.050
F6	7.54±0.31	1.082±0.004	767.67±7.506	83.92±0.72	2.99±0.067	1.173±0.025
F7	5.60±0.15	1.059±0.002	816.33±8.622	79.04±0.97	3.57±0.130	1.127±0.031
F8	6.87±0.75	1.074±0.009	837.00±8.544	76.55±1.41	4.56±0.130	1.060±0.020
F9	8.07±0.27	1.088±0.003	843.67±8.083	74.70±0.83	3.83±0.085	1.013±0.035

***mean ± SD (n = 3)**

Result and discussion:

The floating microspheres of Amoxicillin Trihydrate were prepared by Ionotropic Gelation Method. The results of the physico-chemical characterization are shown in Table 2. The prepared floating microspheres were found to be discrete, spherical and free flowing. All batches show percent entrapment more than 50% and it is found that entrapment of drug increases with an optimum amount of the polymer. Higher amount of the HPMC and Carbopol leads to decrease entrapment of the drug. Formulation F4 shows maximum entrapment whereas formulation F1 shows minimum entrapment of the drug in the polymer as shown in table 2.All batches showed a percentage yield of greater than 88%, whereas four batches showed a yield of more than 90%. Percentage yield is found to be higher with formulation F4.Angle of repose, Hausner ratio, and Carr's index were determined to predict flow ability. A higher Hausner ratio indicates greater cohesion between particles while a high Carr index is indicative of the tendency to form bridges. The prepared microspheres exhibited good flow properties. From the sieve analysis study it was found that the formulations have the size range of 700µm-900µm. The particle size distributions of the formulations F1 to F9 are shown in table 2. Surface morphology characteristics were studied using SEM (Figure 1-4). SEM indicated that the prepared microspheres are spherical with smooth surface; distinct

pores are evident on the surface of microspheres, which will be responsible for the release.

The photomicrographs also showed presence of loose crystals of drug on the surface of few microspheres.

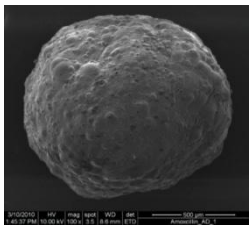

Fig: 1

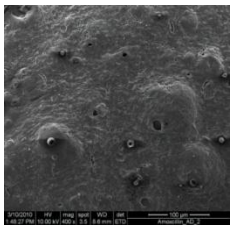

Fig: 2

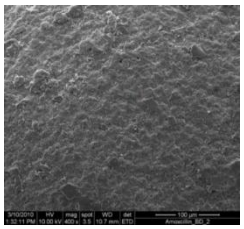

Fig: 3

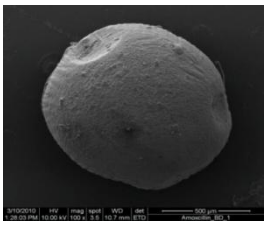

Fig: 4

Fig 1: Scanning Electron Microscopy of Amoxicillin Microsphere of batch F4 before dissolution, Fig 2: Scanning Electron Microscopy of Amoxicillin Microsphere's Surface of batch F4 before Dissolution, Fig 3: Scanning Electron Microscopy of Amoxicillin Microsphere of batch F4 after Dissolution, Fig 4: Scanning Electron Microscopy of Amoxicillin Microsphere's Surface of batch F4 after Dissolution.

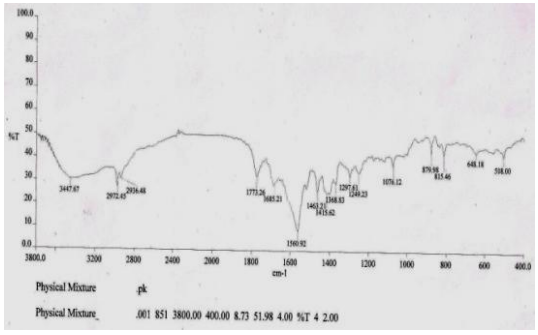

Fig: 5

Fig 5: IR Spectra of Drug (Amoxicillin),

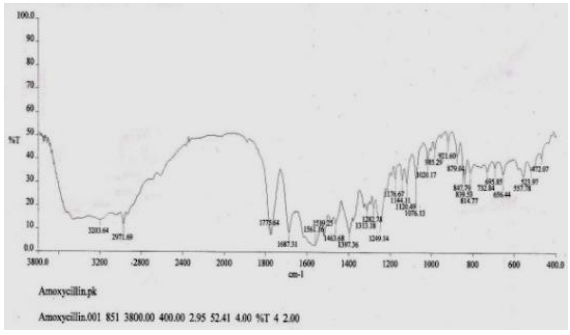

Fig: 6

Fig 6: IR Spectra of Drug+ Polymers (Amoxicillin + HPMC+ Carbopol+ Sodium Alginate)

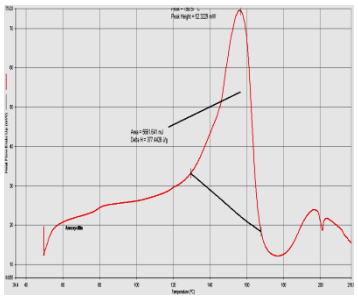

Fig: 7

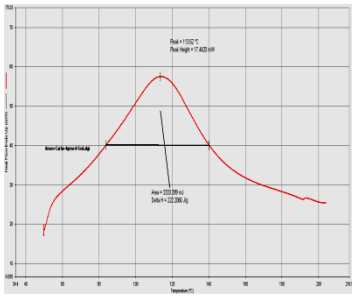

Fig: 8

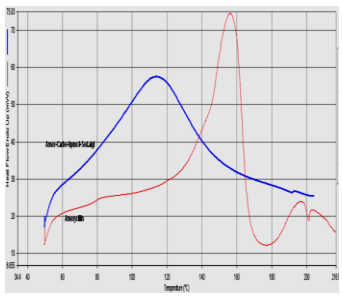

Fig: 9

Fig 7: DSC Thermogram of Drug (Amoxicillin), Fig 8: DSC Thermogram of physical mixture (Amoxicillin + Carbopol + HPMC + Sodium Alginate), Fig 9: Combined DSC Thermogram of Drug and Physical Mixture.

From the floating efficiency study it can be conclude that microspheres floated for prolonged time over the surface of the dissolution medium without any apparent gelation. Buoyancy percentage of the microspheres was in the range of 60 to 90% at the end of 12 h. Formulation F4, F5 and F6 has shown the highest percentage of floating. The nature of the polymer influenced the floating behaviour of the microspheres.

The drug release from floating microspheres for the formulation 91%, 90% and 93% at the end of 10 h for F2, F3 and F6 respectively was found to be satisfactory. The data obtained from in vitro dissolution studies were fitted to zero-order, first-order, Higuchi and Korsemeyer-Peppas equations.The zero-order plots were found to be fairly linear as indicated by their high regression values of the above formulations. To confirm the exact mechanism of drug release, the data were fitted according to Korsemeyer-Peppas equation. Slope values ($0.5<n<1.0$) suggest that the release of Amoxicillin trihydrate from floating microspheres followed non-Fickian diffusion mechanism.

Table 3: Release Data to Various Release Kinetics Models

Formulation code	Zero Order Model		First-Order Model		H-M Model		Korsmeyer-Peppas Model	
	r^2	k_0	r^2	k_1	r^2	k_h	r^2	K_{kp}
F1	0.970	8.706	0.921	-0.088	0.918	36.35	0.935	0.844
F2	0.981	9.624	0.950	-0.120	0.971	41.08	0.989	0.928
F3	0.984	8.430	0.830	-0.089	0.960	35.73	0.973	0.929
F4	0.995	8.817	0.860	-0.114	0.963	37.22	0.987	0.799
F5	0.995	8.308	0.948	-0.087	0.982	35.41	0.997	0.848
F6	0.982	8.008	0.919	-0.077	0.937	33.57	0.969	0.808
F7	0.978	7.989	0.968	-0.086	0.984	34.38	0.983	0.766
F8	0.981	7.510	0.960	-0.087	0.993	32.42	0.993	0.667
F9	0.982	7.812	0.979	-0.073	0.969	33.30	0.967	0.790

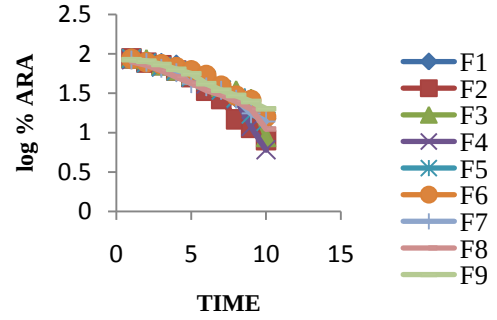

Fig 10: First order release.

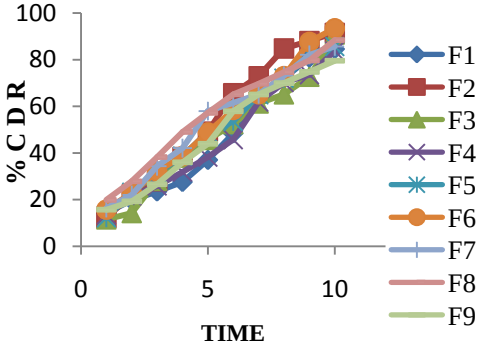

Fig 11: Zero order release.

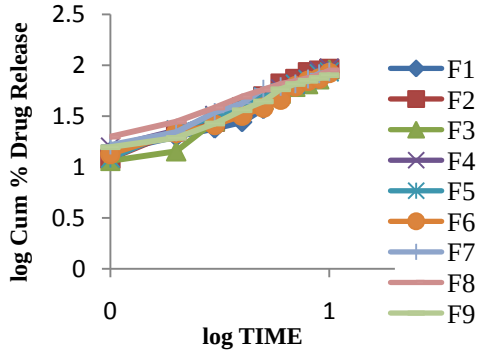

Fig 12: Korsmeyer -Peppas release

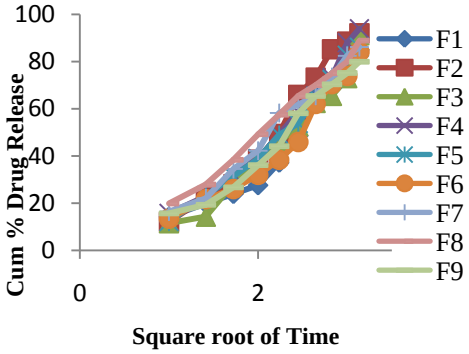

Fig 13: Higuchi release.

CONCLUSION

Hydroxy propylmethyl cellulose K4M,Carbopol 940are selected for formulations of because it is generally regarded as nontoxic, non-allergic and non-irritant polymer. As the success of formulation depends upon, its floating characteristics like duration of floating of microspheres, percentage of floating of micro-spheres as well as in its yield, drug content and en-trapment efficiency. So the technique fulfilling all these requirements out of the used techniques was non aqueous solvent evaporation technique.From this study, it can be concluded that, the formulation retained for longer periods of time in the stomach (spatial control) and provides sustained release of the drug. Hence, Amoxicillin trihydrate floating microspheres retained for longer periods of time in the stomach may leads to improve the therapeutic effect of the drug by increasing its bioavailability.

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