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FORMULATION AND EVALUATION OF FLOATING MICROSPHERES IN COMBINATION OF AMOXICILIN AND SUCRALFATE

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ABSTRACT

The development of oral drug-delivery systems for a specific drug involves the optimization of the dosage form and characteristics of GI physiology. Although significant advances have been made to develop the drug-delivery systems, most of the dosage forms are still designed on an empirical basis. For oral solid-delivery systems, drug absorption is unsatisfactory and highly variable between the individuals despite excellent in vitro release patterns. The main difficulty is the physiological variability such as GI transit in addition to gastric retention time (GRT), as the latter plays a dominating task on the whole transit of the dosage form. Such physiological variability makes it complicated to label the drug-delivery systems with a specific *in vivo* performance even with reproducible *in vitro* data.

KEYWORDS

Amoxicillin, Sucralfate, Microspheres and Optimization.

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INTRODUCTON

The fundamental object of study is to achieve a site-specific or local effect of the drug for an extended period of time. The design of appropriate dosage regimens is a vital element in accomplishing this goal. Oral route is the most favourite route by medical practitioners and manufacturer due to the highest satisfactoriness of patients. Around 60% of all dosage forms are exist as the oral solid dosage form. The oral route has achieved the most concentration and is quite successful. This is because of the ease of administration as well as the fact that gastrointestinal (GI) physiology offers more flexibility in dosage-form design than most other routes. The development of oral drug-delivery

systems for a specific drug involves the optimization of the dosage form and characteristics of GI physiology. Although significant advances techniques have been applied to develop the drug-delivery systems, most of the dosage forms are still developed on an empirical basis. For oral solid drug-delivery systems, drug absorption is not satisfactory and highly variable between the individuals despite excellent in vitro release patterns.

MATERIAL AND METHODS

Preparation of Floating Microsphere of Amoxicillin

Floating microsphere loaded with Amoxicillin and Sucralfate was prepared using emulsion solvent diffusion technique. The Active pharmaceutical ingredients (API) to polymer ratio used to form the different formulations was 1:7. The polymer content was a mixture of Ethyl cellulose Hydroxypropylmethylcellulose (HPMC) as shown in Table No.5. The API and polymer mixture is dissolved in a mixture of ethanol (8ml) and dichloromethane (8ml) was dropped in to 0.75% polyvinyl alcohol solution (200ml). The solution was stirred with a propeller-type agitator at 40°C temperatures for 1 hour at 300rpm. The prepared floating microspheres were passed through sieve no.12 and washed with water and dried at room temperature in a desiccator. Many batches of floating microsphere were formed as follows.

Evaluation of Microspheres

Particle size analysis

Particle size analysis plays an important role in determining the release characteristics and floating property. An optical microscope has been used to measure sizes of floating microspheres and the mean particle size was calculated by measuring nearly 200 particles with the help of a calculated ocular micrometre.

Floating behaviour of Floating microsphere

Prepared floating microsphere were weighted 100mg and placed in 0.1 N HCI. The mixture was stirred with paddle at 100rpm. The layer of floated microspheres was pipetted and followed by filtration at the interwal of 1, 2, 4 and 6 hours. The collected floating microspheres were dried in a desiccator till overnight. The percentage of separated floating microspheres was calculated by using the following equation:

$$\% \text{ Floating microsphere} = \frac{\text{Weight of floating microsphere}}{\text{Initial weight of floating microsphere}} \times 100$$

Drug Entrapment

The various formulations of the floating microspheres were subjected for drug content. Floating microspheres from all batches were accurately 50mg weighed and then crushed. The powdered microspheres were dissolved with ethanol 10ml in 100ml volumetric flask and then make up the volume with 0.1N HCl. This prepared solution is than filtered by using whatman filter paper No. 44. After filtration, 10ml was taken out from filtered solution and make up volume up to 100ml with 0.1N HCl. Again, from this solution 2ml was taken out and add 2ml of Rhodamine B and Extracted with Chloroform and the absorbance was measured at 558 nm against blank. The percentage of drug entrapment was measured as follows.

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

Percentage Yield

The formed floating microspheres of size range 609-874µm were collected and then weighed from different formulations. The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the microspheres.

$$\% \text{ Yield} = \frac{\text{Actual weight of product}}{\text{Total weight of drug and polymer}} \times 100$$

Surface and Shape Characterization of Prepared 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 of drug release were observed for surface morphology and shape by using scanning electron microscope JEOL, JSM-670F Japan. Sample of prepared formulation was fixed by carbon tape and fine gold sputtering was applied in a high vacuum evaporator. During scanning, the acceleration voltage was set at 3.0 KV. Microphotographs were captured on different

magnification and higher magnification (500X) was considered 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 formulated floating microspheres is equivalent to 100mg of drug were dispersed in 900ml of 0.1 N HCI (pH 1.2) maintained at 37 ± 0.5°C temperature and was stirred at 100rpm. 1ml sample was taken at predetermined intervals of time and filtered. Then equal volume of dissolution medium was replaced in the vessel after each withdrawal to maintain sink condition. The collected samples were treated with methyl orange and analyzed spectrophotometrically at 416 nm to determine the concentration of drug present in the dissolution medium.

Drug Release Kinetic Data Analysis

Several kinetic models have been proposed to elaborate the release characteristics of a drug from matrix system. The following three equations are commonly used, because of their simplicity and applicability. Equation 1, the zero-order model equation (Plotted as cumulative percentage of drug released from the formulation verses time); Equation 2, Higuchi's square-root equation (Plotted as cumulative percentage of drug released vs square root of time); and Equation 3, the Korsmeyer-Peppa's equation (Plotted as Log cumulative percentage of drug released vs Log time). To study the release kinetics of Amoxicillin from the floating microspheres the release data was compared with these three equations

Zero order equation

When a graph between cumulative percentage of the drug released from the matrix and time is plotted, zero order release is linear. Such a plot was indicating that the release rate is independent of concentration.

$Q_t = k_0.t$ …… (1)

Where Q_t, is the percentage of drug released at time t and k_0 is the release rate constant;

First order equation

$In (100 - Q_t) = In\ 100 - k_I.t$ ……. (2)

Where k_I is indication the release rate constant;

Higuchi's equation

$Q_t = k_H.t^{1/2}$ …….. (3)

Where K_H is indicating the Higuchi release rate constant.

Korseymeyers-Peppas:

The curves plotted may have different kind of slopes, and hence it is difficult to exactly pin-point which curve follows perfect zero order release kinetics. Therefore, Korsemeyer's equation was used to confirm the kinetics of drug release.

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

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 'n' value is given by the slope of the linear curve. Peppas stated that the above equation could adequately explain the release of solutes from slabs, spheres, cylinders and discs, regardless of the release mechanism. The value of 'n' shows an indication of the release mechanism. If n = 1, the release rate is not time dependent (typical zero order release or case II transport); n = 0.5 for Fickian release (diffusion/ case I transport); and when $0.5 < n < 1$, anamalous (non-Fickian or coupled diffusion/ relaxation) are implicated. Lastly, when n greater than 1 super case II transport is clear. The slope value of log M_t/M_∞ versus log time curve is 'n'.

RESULTS AND DISCUSSION

Physico-chemical properties of amoxicillin

Organoleptic evaluation

Solubility

Solubility studies of Amoxicillin have been done in various solvent such as water, Chloroform, Ethanol, Methanol, and 0.1N HCL solution. We were found that a solubility of Amoxicillin is good in a Methanol solution.

Melting Point determination

Melting point determination of the acquired drug sample was completed because it is a good first sign of purity of the sample since the presence of relatively small amount of impurity can be identified by a lowering as well as widening in the melting point range.

The melting point of the drug sample range of the drug is 194-196^{o}C.

Partition Coefficient measurement

It is the ratio of the equilibrium concentrations of a dissolved substance in a two-phase system consisting of two largely immiscible solvents. In the case of Chloroform and water:

$P_{o/w}$ = Cchloroform/Cwater

The partition coefficient (P) therefore is the quotient of two concentrations and is usually given in the form of its logarithm to base 10 (log P).

The partition coefficient again defined as ratio of concentrations of unionized compound in between the two solutions. To determine the partition coefficient of ionisable solutes, the pH of the aqueous phase is adjusted so that the predominant form of the compound is un-ionized. log P is the logarithm of the ratio of the concentrations of the un-ionized solute in the solvents:

$$log\ P_{oct/wat} = log\left(\frac{[solute]_{octanol}}{[solute]^{un-ionized}_{water}}\right)$$

Identification test by FTIR

Identification of Amoxicillin by FTIR Spectroscopy with respect to marker compound.

Compressibility Index (%)

$$C.I. = \frac{100\ (V_0\text{-}V_f)}{V_0} \quad OR\ C.I. = \frac{\text{Tapped density- Bulk densities}}{\text{Tapped density}} x100$$

The compressibility index of Amoxicillin was found to be 19.77%.

Hausner ratio

Hausner Ratio = Tapped density / Bulk Density, the Hausner ratio of Amoxicillin was found to be 1.24**.**

Angle of Repose

Procedure

The angle of repose is a relatively simple technique for estimating the flow ability of a powder through a funnel and fall freely onto a surface. The height and diameter of the resulting heap is observed and put the value in the equation, the angle of repose can be calculated. Weigh 10gm of Amoxicillin powder accurately, and pass through the fennel height up to 10cm from surface and measure the height and diameter by scale.

$\text{Tan}\ \theta = h/r$

Where h, r is the relatively height and radius of the powder concentration, The Angle of repose of Amoxicillin is 40.57 degree. Particle size pass through 40# is 100 (%w/w).

Moisture by Karl-Fischer Apparatus (KF)

The Moisture content of Amoxicillin is 0.81%

DETERMINATION OF λ_{maX} BY UV-VISIBLE SPECTROSCOP

Accurately weighed 10 mg of Amoxicillin separately and dissolved in 10ml of 0.1N HCL in 10ml of volumetric flask and prepared suitable dilution to make it to a concentration of 10µg/ml make adequate of sample with concentration range of 10-50µg/ml Amoxicillin and calculate the spectrum of this solution was run in 200-400nm range in U.V spectrophotometer.

The λ_{max} found for Amoxicillin is 238.0 nm as shown in Figure No.3.

EVALUATION OF AMOXICILLIN FLOATING MICROSPHERES

Particle size analysis

Particle size was calculated by using Optical microscopy method. It is important parameter of floating ability and release of drug from Microsphere. If size of Microspheres is less than 500 µm release rate of drug will be high and floating ability will reduce, white Microspheres ranging between 200µm - 500µm, the floating ability will be good and release rate will be in continuous or sustained manner.

The mean particle size of Amoxicillin microsphere was in range 210 - 264µm as shown in Table No.5.

Floating behaviour of microsphere

Amoxicillin Microsphere was dispersed in 0.1 HCl as simulate gastric fluid. Floating ability of different formulation was found not same. It was differed according to EC 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 (66.12-45.09%). The floating ability of microsphere is decreased by increasing the HPMC ratio.

Drug Entrapment

The drug entrapment efficacies of different formulations were in range of 48.47 - 74.19% w/w. Drug entrapment efficacy slightly decrease with increase HPMC content and decreased EC ratio in Microspheres. This is because of the permeation characteristics of HPMC that it could facilitate the diffusion of part of entrapped drug to surrounding

medium during preparation of Amoxicillin microspheres.

Percentage Yield

Percentage yield of different formulation was calculated by weighing the prepared floating microspheres after drying. The percentage yield of different formulation was found in between of 56.84 - 82.87%.

Scanning Electronic Microscopy

Shape and surface characteristic of floating microspheres was examined by Scanning Electronic Microscopy analysis (SEM). Surface morphology of formulation examines at different magnification, which illustrate the smooth surface of floating Microspheres.

***IN-VITRO* DRUG RELEASE STUDY**

Drug Release Studies

***In vitro* drug release study of Amoxicillin loaded Floating Microsphere**

Comparative release study of all formulation

The *In vitro* drug release data of the optimized formulation of floating microsphere has been subjected to goodness of fit test by linear regression analysis according to zero order, first order kinetic equation, Higuchi's and Korsmeyer's models in respect to determine the mechanism of drug release. When the regression coefficient values of were compared, it was observed that 'r' values of Higuchi were maximum i.e 0.954 hence indicating drug release from formulations was found to follow Higuchi kinetics.

Table No.1: Formulations of the floating microspheres prepared

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

Table No.2: Organoleptic property of Amoxicillin

Color	:	White to almost white crystalline powder
Odor	:	Odourless
Taste:	:	Bitter

Table No.3: Solubility studies of Amoxicillin in different solvent

S.No	Solvent used	Solubility
1	Water	Slightly Soluble
2	0.1 N HCL	Soluble
3	Ethanol	Slightly Soluble
4	Methanol	Freely Soluble
5	0.1N NaOH	Soluble

Table No.4: Calibration curve of Amoxicillin

Replicate	10	20	30	40	50
1	0.145	0.285	0.418	0.561	0.702
2	0.143	0.283	0.419	0.562	0.703
3	0.145	0.286	0.418	0.561	0.702
Mean	0.144	0.285	0.418	0.561	0.702
S.D.	0.001	0.002	0.001	0.001	0.001
% RSD	0.800	0.537	0.138	0.103	0.082

Stastical data for linearty

S.No	Parameter	Remark
1	Linearty Range	10-50 µg/ml
2	Regression Equation	0.014x+0.001
3	Correlation Cofficient	0.999

Table No.5: Mean particle size of different batches of amoxicillin microsphere

S.No	Formulation code	Mean particle size (µm)
1	F_1	212±12
2	F_2	225±21
3	F_3	264±23
4	F_4	236±25
5	F_5	242± 24
6	F_6	244±40
7	F_7	210±23

Table No.6: Comparative Release Study data of formulation F1-F7

S.No	Time (hr)	% Of Drug Release						
		F1	F2	F3	F4	F5	F6	F7
1	0.5	16.429	15.000	14.286	14.286	17.857	16.429	14.286
2	1.0	26.536	18.607	18.571	18.571	28.036	25.821	22.857
3	1.5	30.679	27.357	27.321	27.321	34.393	31.357	33.964
4	2.0	57.107	32.929	32.893	32.893	43.857	43.536	39.143
5	3.0	71.214	60.143	40.821	40.821	61.571	54.821	58.786
6	4.0	81.607	77.214	52.643	52.643	71.500	78.000	56.464
7	6.0	95.214	85.714	72.107	72.107	78.214	93.643	66.036
8	8.0	100.036	90.179	86.714	86.714	95.107	99.893	95.250

Table No.7 Release Kinetics of Optimized Formulation F-7

S.No	Time (Hrs.)	% CDR	Log T	Root T	Log % cum. drug remain to be release	Log cum. % drug release
1	0.5	14.286	-0.301	0.707	1.933	85.714
2	1	22.857	0.000	1.000	1.887	77.143
3	1.5	33.964	0.176	1.225	1.820	66.036
4	2	39.143	0.301	1.414	1.784	60.857
5	3	58.786	0.477	1.732	1.615	41.214
6	4	56.464	0.602	2.000	1.639	43.536
7	6	66.036	0.778	2.449	1.531	33.964
8	7	95.25	0.903	2.828	0.677	4.75

Table No.8: Comparative study of regression coefficient for selection of optimised Formulation F7

S.No	Zero order		First order	Higuchi	Korsmayer
1	r2	0.931	0.831	0.954	0.972

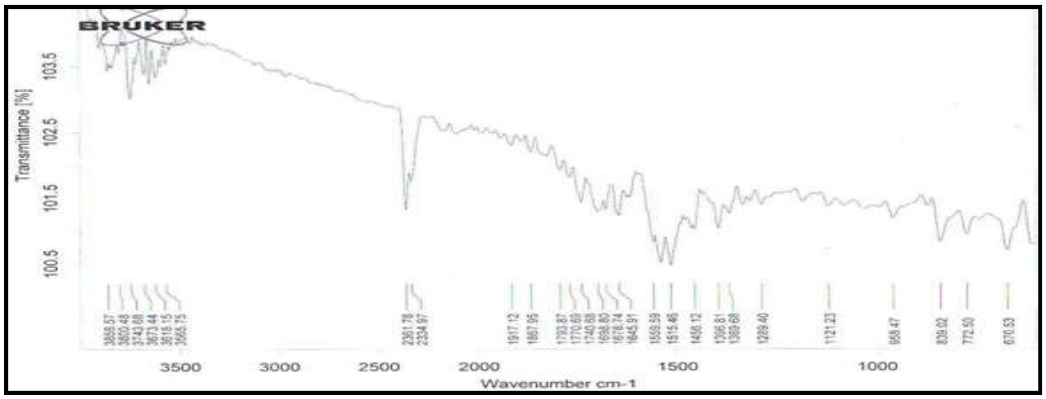

Figure No.1: FT-IR Spectrum of Pure Drug (Amoxicillin)

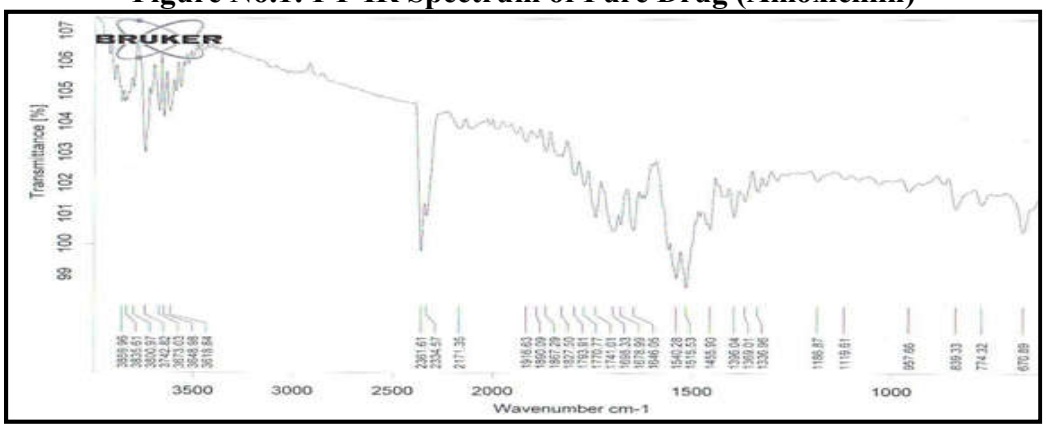

Figure No.2: FT-IR Spectrum of Pure Drug (Amoxicillin + All Excipients)

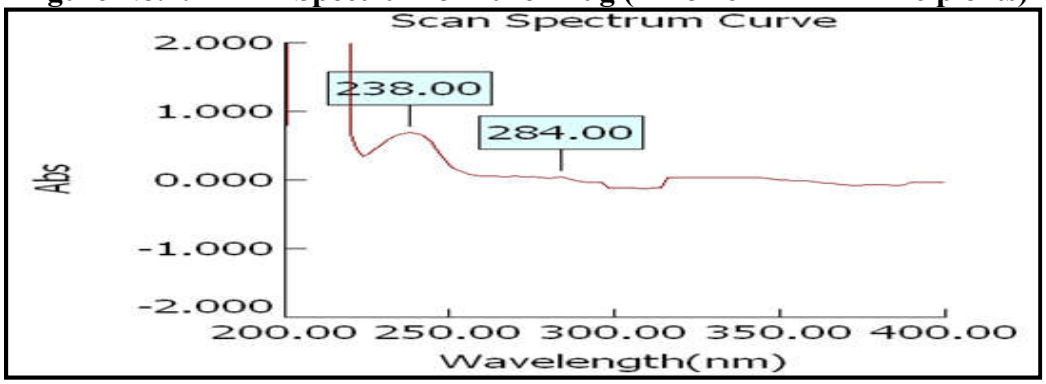

Figure No.3: Determination of λ_{max} of Amoxicillin

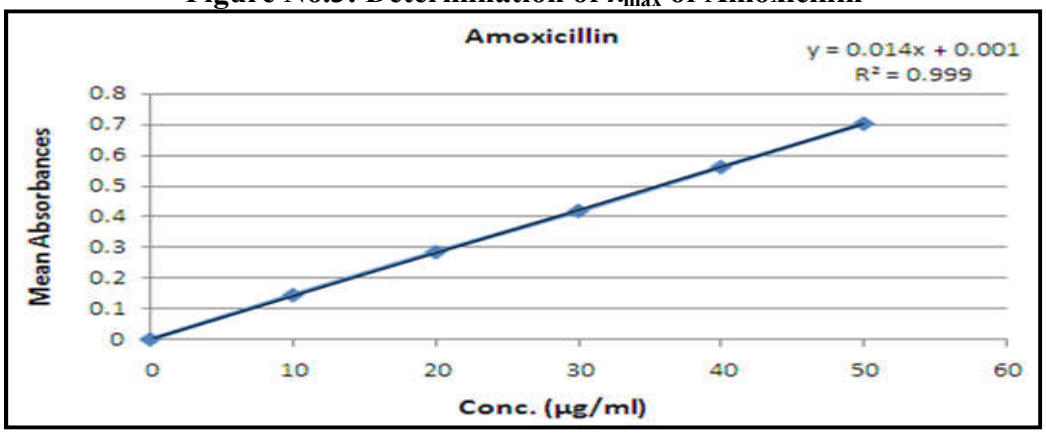

Figure No.4: Calibration Curve of Amoxicillin at 238 nm

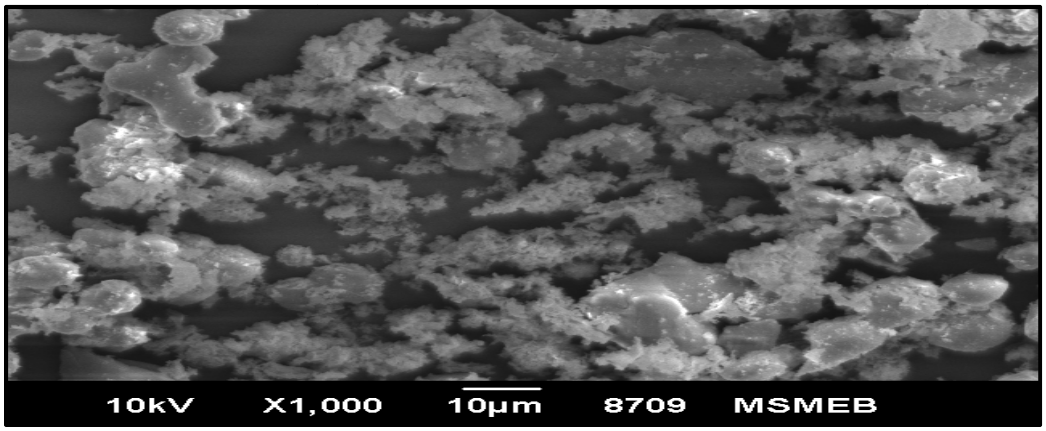

Figure No.5: Scanning Electronic Microscopy Image of Optimized Formulation F-7

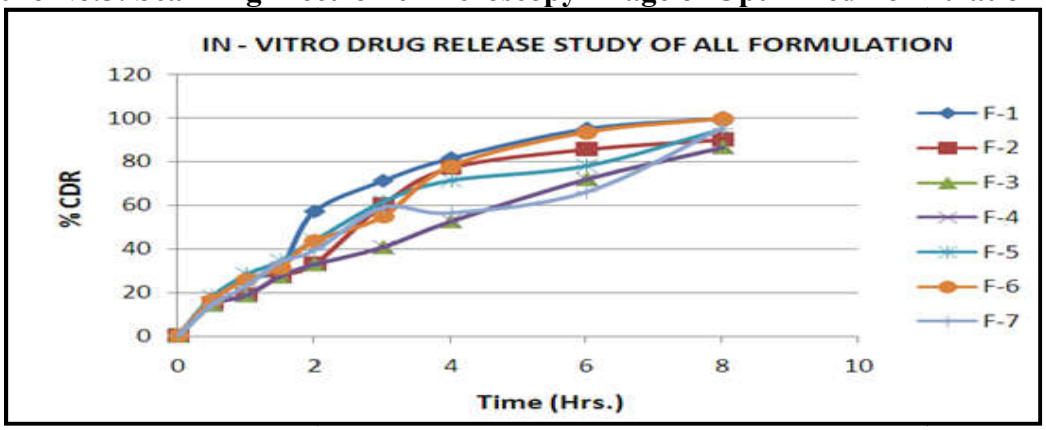

Figure No.6: Graph of release study of formulation F1-F7

CONCLUSION

Drug absorption in the alimentary tract is a highly changeable operation. Floating microspheres are assurance to be potential approaches for gastric retention improve the bioavailability and controlled delivery of various active pharmaceutical ingredients. Great endeavours have been made worldwide to investigate these systems according to patient reliability and requirements, in terms of both therapeutic efficacy and compliance.

Floating microspheres precisely control the release rate of active pharmaceutical ingredients to a specific site and facilitate an enormous impact on health care system. These systems of novel drug delivery also provide tremendous chances in the designing of new controlled and delayed release oral formulations, thus extending the frontier of futuristic pharmaceutical development. Likewise, recent innovations in pharmaceutical research will surely provide real expectations for establishment of novel and effective means in the development of these promising drug delivery systems.

In-vitro data obtained for floating microspheres of Amoxicillin showed good incorporation efficiency, good buoyancy and prolonged drug release. Floating microspheres of different size, shape and drug content could be obtained by varying the formulation variables. From the results it can be concluded that the drug release from the floating microspheres controlled by the polymer proportion. Prepared floating microspheres showed best appropriate balance between floating behaviour and rate of drug release.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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