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Development and Characterization of Floating Microspheres of Acyclovir As Gastroretentive Dosage Form

Srikrishna.T[1]*, P.V.Prasad[1], Md.Nayeem, [1] Sk.Mubashira[1], M.Sai Giridhar[1]
1.Department of Pharmaceutics, Narayana Pharmacy College, Chinthareddypalem, Nellore-524002 A.P., India

ABSTRACT

In the present study, an attempt has been made to prepare floating microspheres of Acyclovir designed as gastroretentive dosage form for the Herpes simplex virus infection as well as vericella zoster infection. The floating microspheres were prepared using Ethylcellulose by Non-aqueous Solvent Evaporation technique which offers advantage of short processing time and gives high encapsulation efficiency. Formulations were characterized for their particle size, practical yield, percentage entrapment efficiency, buoyancy, drug-polymer compatibility (FTIR), Scanning electron microscopy (SEM), in vitro drug release and model fitting kinetics. SEM analysis shows that spherical microspheres with porous surface were formed. The particle size of microspheres was in the range of 262.6±2.52 to 348±2.49µm. Percentage encapsulation efficiency was between 71.6±0.53 to 91.6± 0.32%. Microspheres remained buoyant for more than about 12 h. The results of FTIR spectroscopy indicated the stable character of acyclovir in microspheres and also revealed absence of drug-polymer interaction. The formulation F5 showed results of in vitro drug released (95.56%) and acyclovir microspheres showed release from slow to sustained for more than 10hr. The release obeys zero order model. All the stability studies for the formulation F5 showed no significant change in the percentage drug release studies and percentage buoyancy. The results of factorial batches revealed that the concentration of ethyl cellulose and stirring speed significantly affected drug encapsulation efficiency and particle size of the microspheres. Thus we can conclude that floating microspheres can successfully be developed to sustain the drug release.

Keywords: Floating microspheres, buoyancy, Solvent Evaporation technique, Ethyl Cellulose, *in vitro* release.

*Corresponding Author Email: srikrishna.nlr@gmail.com
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INTRODUCTION

To develop oral drug delivery systems, it is necessary to optimize both the residence time of system within the gastrointestinal tract and release of drug from the system[1, 2]. Gastric emptying of dosage form is an extremely variable process and ability to prolong and control the emptying time is a valuable asset for dosage form. Gastroretentive systems can remain in the gastric region for several hours and hence significantly prolong the gastric residence time of the drug and improves bioavailability, reduces drug waste, improves solubility of drug that are less soluble in high pH environment[3, 4]. The controlled gastric retention of solid dosage forms may be achieved by the mechanism of mucoadhesion, flotation, sedimentation, expansion, modified shape system or by the simultaneous administration of pharmacological agents that delay the gastric emptying[5, 6]. Acyclovir, BCS class III/ IV drug, is widely used in the treatment of Herpes simplex virus infection as well as vericella zoster infection[7, 8]. Its short biological half life (2.5-3.3 h) and low absorption (15-30% of administered dose) only from upper part of GIT are the two major reasons for the need of development of novel drug delivery system. Hence, it was aimed to develop gastro retentive system of acyclovir which results in to complete absorption and higher bioavailability[9, 10].

MATERIALS AND METHOD

Materials:

Acyclovir was a Gift Sample from Doctors Life Sciences Pvt. Ltd. (India). Ethyl cellulose (EC) Purchased from Loba chemie (P) Ltd. Mumbai. Acetone, Soft Liquid Paraffin, Span-80, n-Hexane, Tween-80, Hydrochloric Acid Were from SD Fine Chemicals Ltd, Mumbai. All Other Chemicals used in the study were of AR grade.

Method of Preparation of Floating Microspheres of Acyclovir:

The Floating microspheres loaded with Acyclovir were prepared by using Non-aqueous Solvent Evaporation technique. The drug and ethyl cellulose (18-22cps) mixture was mixed in acetone at various ratios. The slurry was slowly introduced into 90 ml of liquid paraffin while being stirred at constant rpm by a mechanical stirrer equipped with a three bladed propeller type Remi stirrer at room temperature .The solution was stirred for 2hr to allow the solvent to evaporate completely and the microspheres were collected by filtration .The microspheres were washed repeatedly with n-hexane until free from oil. The collected microspheres were dried for 1hr at room temperature and subsequently stored in a desiccator over fused Calcium chloride[11,12]. Different ethyl cellulose: Acyclovir ratios (1:1, 1:2, 1:3, and 1:4) were used in order to

investigate the effect of polymer/drug ratio on release, entrapment efficiency, floating ability and physical characterization of floating microspheres. The effects of stirring speed (800, 1000, 1200 rpm) on floating microspheres were investigated. The various batches of floating microsphere were prepared as follows.

Table 1: Composition of Acyclovir Floating Microspheres

S.No	Formulation Code	Acyclovir (gm)	Ethyl Cellulose (gm)	Acetone (ml)	Volume of soft liquid paraffin(ml)	Stirring speed (rpm)
1	F1	0.2	0.1	3	90	1000
2	F2	0.2	0.2	6	90	1000
3	F3	0.2	0.3	9	90	1000
4	F4	0.2	0.4	12	90	1000
5	F5	0.2	0.4	12	90	800
6	F6	0.2	0.4	12	90	1200
7	F7	0.2	0.4	12	180	1000
8	F8	0.2	0.4	12	270	1000

Evaluation of Acyclovir Floating Microspheres:

Micromeritic properties:

The microspheres were characterized by their micromeritic properties such as particle size, bulk density, tapped density, compressibility index, Hausners ratio and angle of repose

Particle size:

The particle size was measured by microscopic technique13.

Bulk density:

In this method floating microspheres are transferred to a measuring cylinder and is tapped manually till a constant volume is obtained. This volume is bulk volume and it includes true volume of the powder and the void space among the microspheres.

$$\text{Bulk density} = \frac{\text{Mass of microspheres}}{\text{Bulk volume}}$$

Tapped density:

In this method floating microspheres were transferred to a measuring cylinder & tapped for 100 times. After tapping volume of microspheres was visually examined. The ratio of mass of microspheres to volume of microspheres after tapping gives tapped density floating microspheres[14].

$$\text{Tapped density} = \frac{\text{Mass of microspheres}}{\text{Volume of microspheres after tapping}}$$

Percent Compressibility index was determined by using the formula,

$$\% \text{ Compressibility index} = 1 - \frac{V}{V_o} x100$$

Here V and Vo are the volumes of the sample after and before the standard tapping, respectively.

Hausner's ratio:

Hausner's ratio of microspheres was determined by comparing tapped density to bulk density using the equation.

$$\text{Hausners ratio} = \frac{\text{Bulk density}}{\text{Tapped density}}$$

Angle of repose:

It is a technique to determine the flow property of microspheres. In this technique microspheres were filled in the funnel and were dropped from a specific height. The height and radius of the generated cone to calculate the angle[14, 15].

$$\boldsymbol{\theta = \tan^{-1} h / r}$$

Where,

θ - Angle of repose

h - Height of granules above the flat surface

r - Radius of the circle formed by the granule heap.

Percentage Yield:

The percentage yield of different formulations was determined by weighing the floating microspheres after drying and comparing with total weight of the drug and polymer required to formulate those formulations. The percentage yield was calculated as follows.

$$\% \text{ yield} = \frac{\text{Actual weight of product}}{\text{Total weight of excipient and drug}} \text{ x } 100$$

***In vitro* Buoyancy:**

Floating microspheres (50mg) were placed in 0.1N HCl (100ml) containing Tween 20 (0.02 W/V %). The mixture was stirred at 100 rpm in a magnetic stirrer. The time taken by the microspheres start floating was noted down as floating lag time and the floating ability of microspheres were observed up to 12hr[16].

Incorporation efficiency:

The various batches of the floating microspheres were subjected to estimation of drug content. The Floating microspheres equivalent to 50mg of Acyclovir from all batches were accurately

weighed and crushed. The powdered of microspheres were dissolved ethanol (5ml) in volumetric flask (100ml) and made the volume with 0.1N HCl. This solution is filtered through Whattman filter paper, diluted suitably and analyzed for drug content spectrophotometrically at 255 nm using 0.1N HCl as blank.

Scanning electron microscopy:

Scanning electron microscopy was carried out for best formulation Dry microspheres were placed on an electron microscope brass stub coated with gold in an ion sputter. Then picture of microsphere were taken by random scanning of the stub. The SEM analysis of the microspheres was carried out by QUNTA-200 FEI (Netherland) using analytical scanning electron microscope. The microspheres were viewed at an accelerating voltage of 20KV.

***In vitro* Release Studies:**

In vitro dissolution study was performed in paddle type dissolution apparatus assembly. Accurately weighed microspheres equivalent to 100 mg drug were taken in a gelatin capsule and placed in dissolution vessel. Dissolution study was carried out in 900 ml 0.1N HCI (pH 1.2) maintained at 37 ± 0.5°C and stirred at 100 rpm. 5 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 analyzed spectrophotometrically at 255 nm to determine the concentration of drug present in the dissolution medium[17].

Kinetic Studies:

The in vitro release data obtained was treated to zero order kinetics, first order kinetic, higuchi model and korsemeyer peppas model to know precisely the mechanism of drug release of the microspheres.

RESULTS AND DISCUSSION

Standard Calibration Curve:

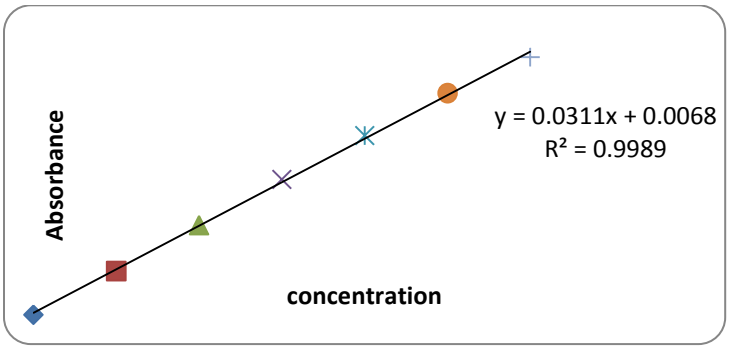

Figure 1: Calibration Curve of Acyclovir using 0.1N HCl

Calibration curve for the estimation of Acyclovir was constructed in 0.1N HCl at 255 nm, as shown in Figure 1. The method obeyed Beer's Lambert law in the range of 5 to30 mcg/ml.

Compatibility Study:

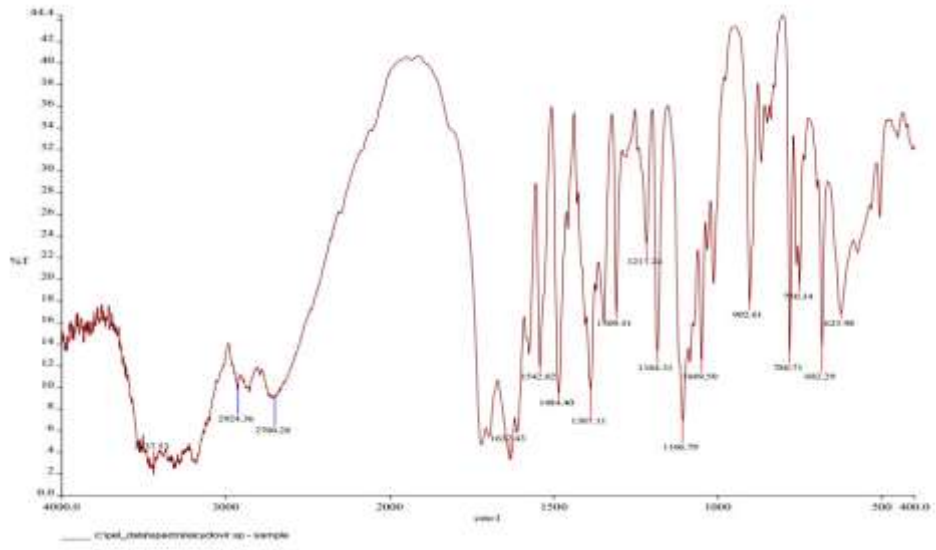

Figure 2: FTIR Graph of Acyclovir

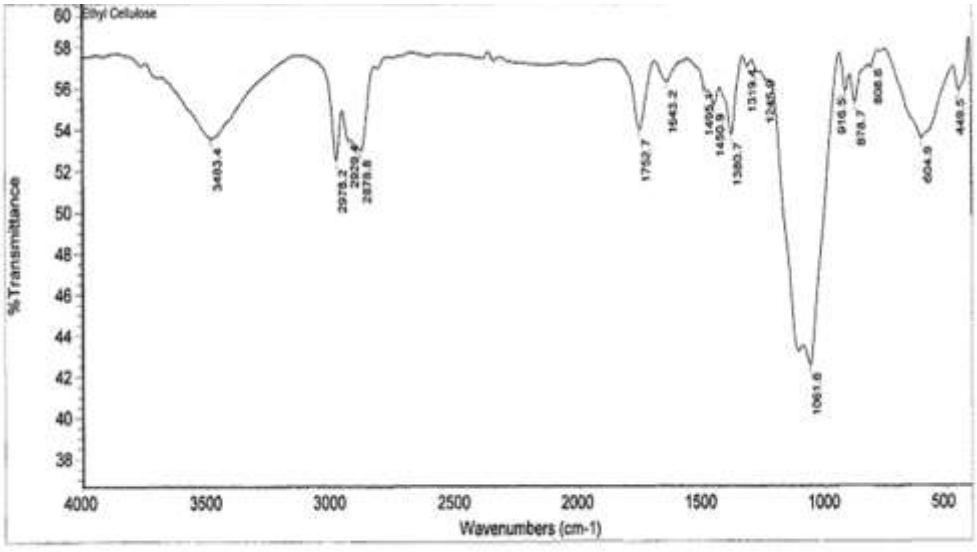

Figure 3: FTIR Graph of Ethyl Cellulose

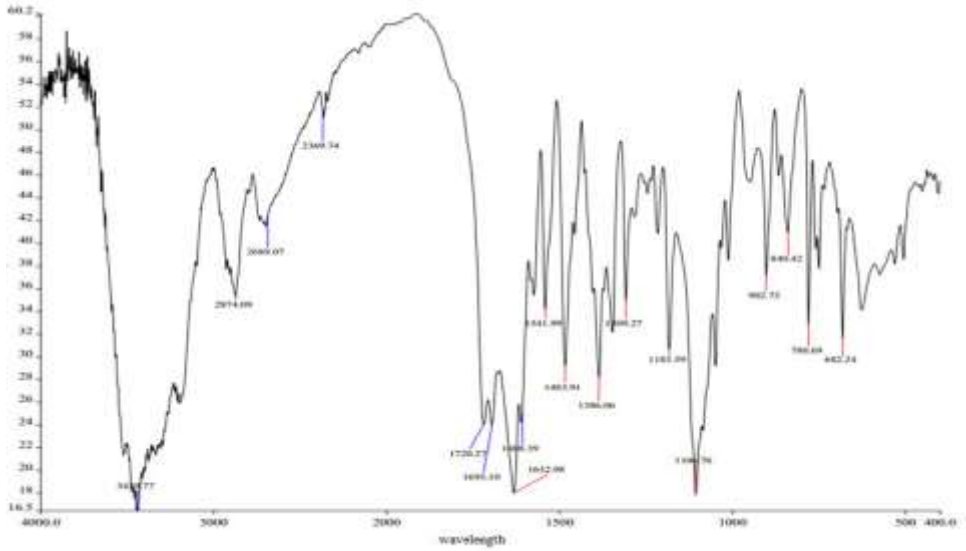

Figure 4: FTIR Graph of Acyclovir + Ethyl Cellulose

The compatibility study was performed using FTIR for drug polymer mixtures. From the FTIR graphs of drug- polymer mixture, it was found that similar peaks of the drug are available. So, it proves that there is no compatibility with the polymers.

Table 2: Micromeritic properties

Formulation code	Mean Particle Size (µm)	Tapped density (gm/cm^3)	Carr's index	Hausner's ratio	Angle of repose	Bulk density (gm/cm^3)
F1	262.6±2.52	0.334±0.53	7.94±1	1.07±0.03	26.5±2.2	0.290±0.5
F2	273.8±2.54	0.253±0.51	7.96±1.7	1.08±0.02	28.2±2.1	0.201±0.1
F3	283.2±3.23	0.314±0.58	8.82±1.3	1.09±0.02	25.3±2.8	0.251±0.1
F4	398.3±3.41	0.300±0.57	6.57±1.5	1.07±0.03	28.0±1.9	0.254±0.1
F5	317.8±2.12	0.232±0.56	6.32±1.6	1.06±0.02	29.4±2.2	0.292±0.5
F6	329.3±3.26	0.234±0.55	5.83±1.2	1.06±0.02	24.6±2.5	0.297±0.5
F7	337.6±1.95	0.288±0.49	6.16±1.5	1.07±0.03	28.6±2.3	0.242±0.1
F8	348.3±2.49	0.327±0.5	10.3±1	1.11±0.02	28.8±2.1	0.252±0.1

The mean particle size of the microspheres formulation F1 to F8 containing ethyl cellulose is in the range of 262.6±2.52 to 348±2.49 respectively (as shown in table 2). The effect of polymer concentration on the particle size of microspheres was determined. The mean particle size of the microspheres was found to increase with increasing ethyl cellulose concentration (as shown in Table.2). The viscosity of the medium increases at a higher ethyl cellulose concentration resulting in enhanced interfacial tension. Shearing efficiency is also diminished at higher viscosities. This results in the formation of larger particles.

When the stirring speed was decreased from 1200 to 800 rpm the particle size was also decreased (as shown in table 2), it may be due to increase in the viscosity of polymer solution. Also, when

the volume of liquid paraffin decreased, the polymer concentration increased and particle size was increased. The bulk density, tapped density, Hausner's ratio of formulations F1 to F8 containing ethyl-cellulose polymer & formulation was in the range of 0.201 + 0.1 to 0.290 + 0.50 gm/cm^3.The Carr's index of formulation F1 to F8 containing ethyl cellulose was in between 6.32 ± 1.6 to 10.32+ 0.1 respectively. The angle of repose of formulation F1 to F8 containing ethyl-cellulose & formulation was in the range 24.6 ± 2.5 to 29.68 + 1.2 respectively (as shown in table). The values of Carr's index and angle of repose indicate good flow properties.

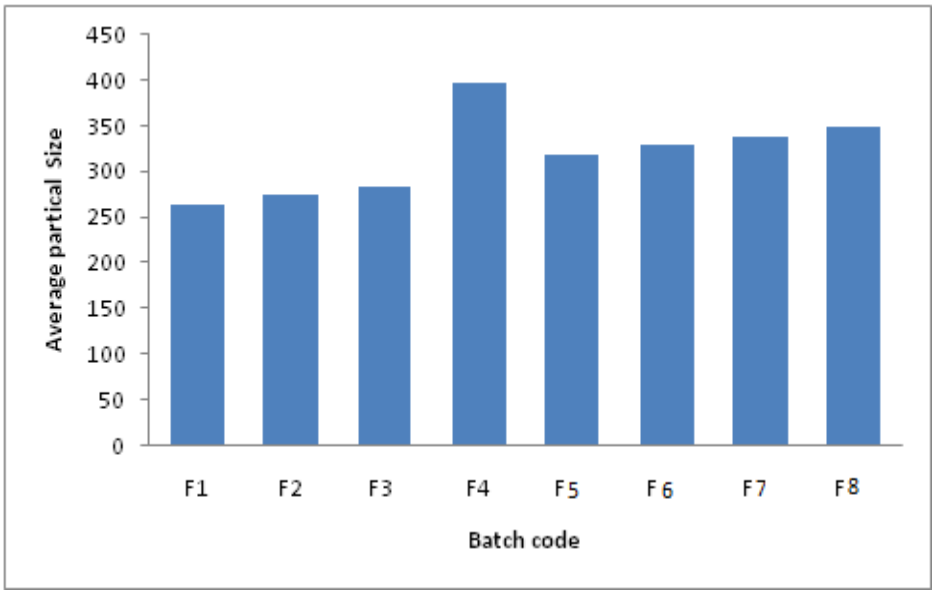

Figure 5: Graph of Average Particle Size

Table 3: *in-vitro* buoyancy, Percentage yield and incorporation efficiency

Formulation code	In vitro buoyancy (%) for 12 hrs.	Percentage yield	Incorporation efficiency (%)
F1	80.66±1.24	76.2±0.53	77.4±0.23
F2	84.93±1.36	81.7±0.51	83.7±0.61
F3	86.69±1.62	88.6±0.58	87.6±0.10
F4	89.34±1.48	94.3±0.57	91.6±0.32
F5	91.89±1.91	87.0±0.56	88.4±0.46
F6	83.93±1.10	87.2±0.55	81.2±0.59
F7	80.33±1.01	85.8±0.49	71.6±0.53
F8	78.21±1.24	86.6±0.50	66.6±0.23

Percentage Yield:

The percentage yield of floating microsphere formulation F1 to F8 containing ethyl-cellulose was in range 76.2±0.53 to 94.3±0.53 (as shown in table 3). To observe the effect of polymer concentration on the percentage yield of the floating microspheres, formulations were prepared at varying concentration of ethyl cellulose (as shown in table 3).The yield of the floating

microspheres increased with increasing polymer concentration. At low concentration of ethyl-cellulose part of the polymer solution aggregated in a fibrous structure, as it solidified prior to forming droplets or the transient droplets were broken before solidification was complete due to poor mechanical strength resulting into low yield. There was no significant effect of processing medium and stirring speed on percentage of the microspheres.

***In-vitro* buoyancy:**

The purpose of preparing floating microspheres was to extend the gastric residence time of a drug. The buoyancy test was carried out to investigate the floatability of the prepared microspheres. The microspheres were spread over the surface of a simulated gastric fluid and the fraction of microspheres buoyant and settled down as a function of time was quantitated. The *In vitro* buoyancy of formulation F1 to F8 containing ethyl-cellulose was in range from 80332±1.0 to 91.89± 1.91 respectively (as shown in table 3). Among all formulation F3a was found to be highest *in-vitro* buoyancy 91.89±1.91. As the stirring speed was decreased the particle size was decreased. The larger the particle size was formed, which was floated for longer time. There was no significant effect of volume on *in vitro* buoyancy.

Incorporation efficiency:

The incorporation efficiency of formulation F1 to F8 containing ethyl-cellulose was in the range of 71.6±0.53 to 91.6± 0.32 respectively (as shown in table 3). Among all formulation F4, 91.6±0.32 results demonstrated that increase in concentration of ethyl-cellulose increased the entrapment of the drug. The volume of liquid paraffin significantly influenced the entrapment efficiency of the drug loaded microspheres .As the volume of processing medium was increased from 90 ml to 270 ml the entrapment efficiency was further decreased as shown 91.6± 0.32 to 71.6±0.53 respectively. The reason may be the higher amount of drug extraction into the processing medium, resulting in lower entrapment efficiency. The entrapment efficiency was also influenced with changing the stirring speed. The highest entrapment efficiency was observed with the stirring speed of 1000 rpm. The change of stirring speed from 1000 to 800 rpm and 1200 rpm significantly decreases the entrapment efficiency.

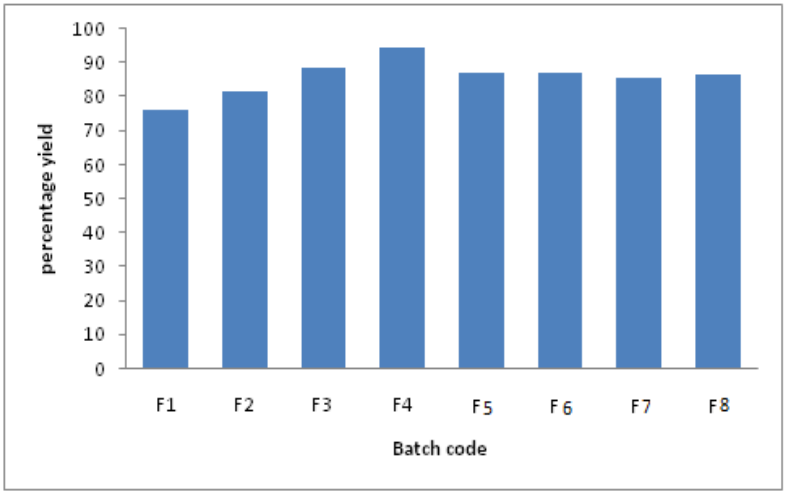

Figure 6: Graph of Percentage Yield

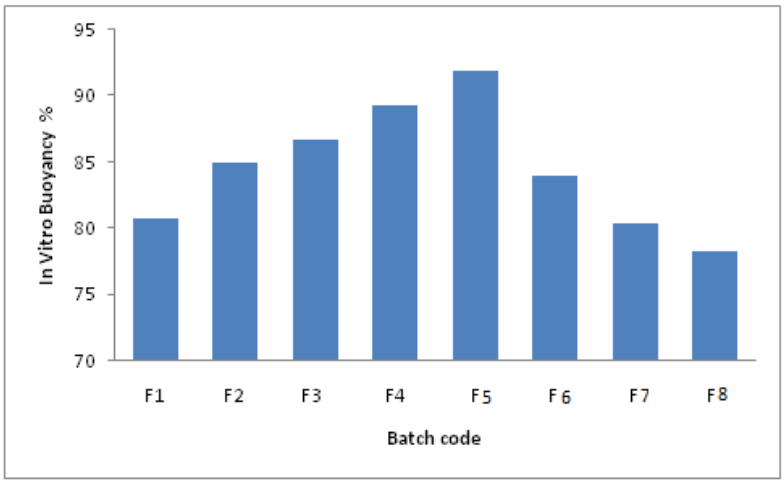

Figure 7: Graph of In vitro buoyancy (%)

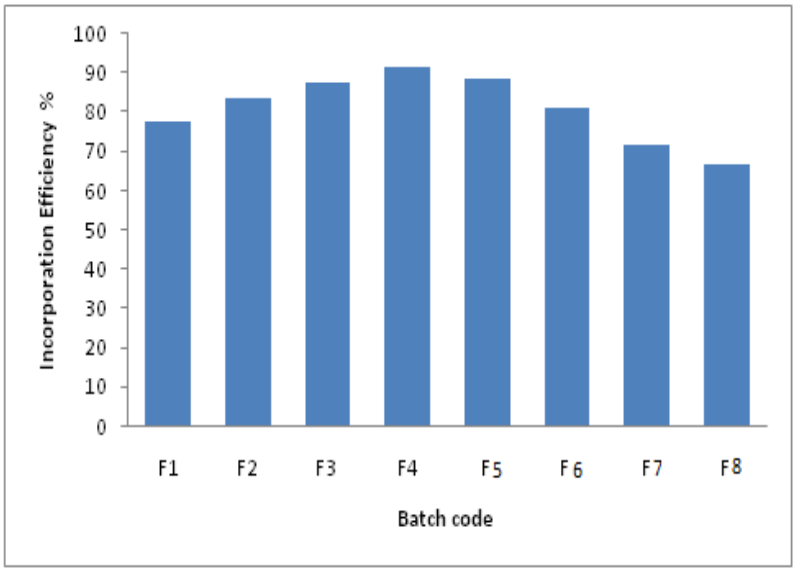

Figure 8: Graph of Incorporation Efficiency (%)

Scanning electron microscopy (SEM) Analysis:

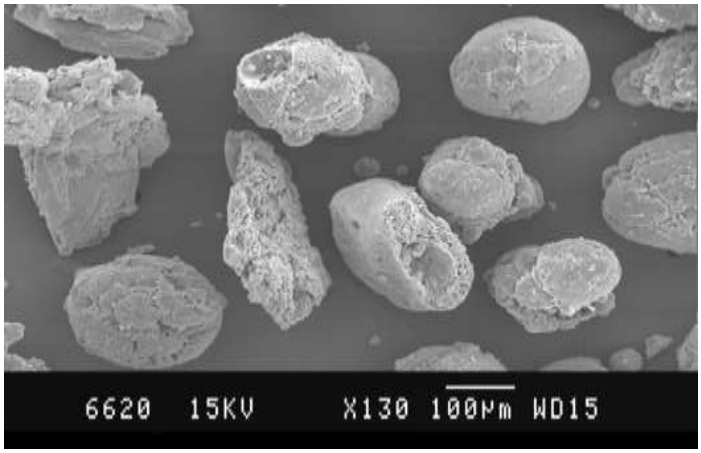

Figure 9: Scanning Electron Microphotographs of Acyclovir Microspheres (Particle Size Range Observed)

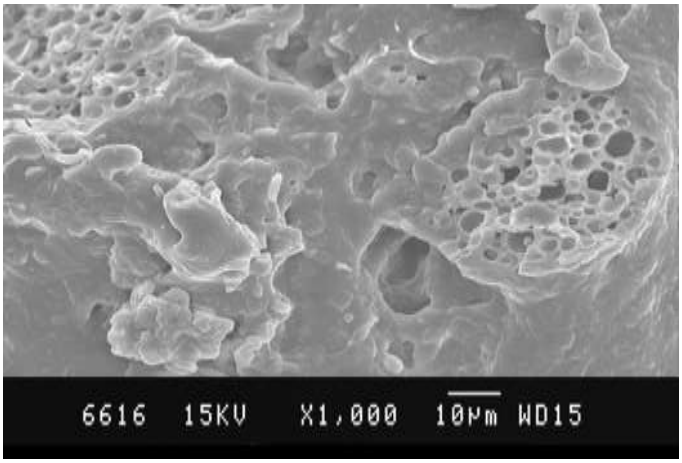

Figure 10: Cross Section showing a Porous Network (microsponges)

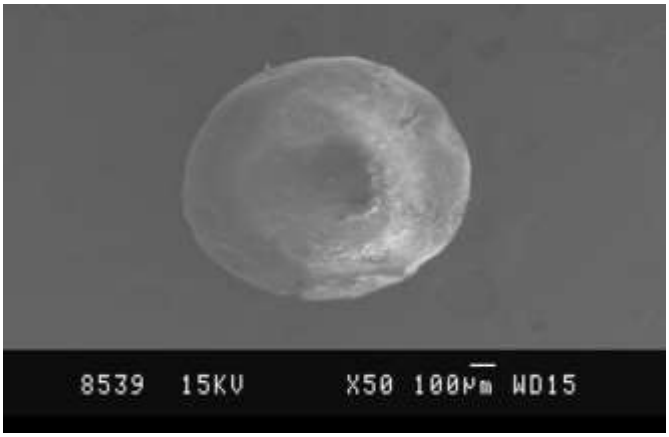

Figure 11: Smooth Surface Observed

Morphology of microspheres was examined by scanning electron microscopy. The view of the microspheres showed a hollow spherical structure with a smooth surface morphology (Figure 11) .Some of the microspheres showed a dented surface structure but they showed good floating

ability on the surface of the medium, indicating intact surface. The outer surface of the microspheres was smooth and dense, while the internal surface was porous. The shell of the microspheres also showed some porous structure (Figure 10). It may be caused by the evaporation of solvent entrapped within the shell of microspheres after forming a smooth and dense skin layer. The transverse section of the prepared microspheres (Figure 10) indicates that the microspheres were hallowed.

Table 4: *In-vitro* drug release profile

Time (hr)	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	15.69	23.16	16.58	14.01	35.69	10.37	20.31	35.63
2	23.32	29.11	21.29	17.69	39.21	18.01	25.31	39.02
3	35.94	34.88	25.91	21.94	44.46	26.98	29.29	44.41
4	41.60	41.90	31.77	27.60	49.82	35.33	34.79	49.80
5	49.45	47.32	36.48	31.64	53.27	45.21	41.28	53.21
6	55.14	52.03	54.91	41.30	57.96	49.19	47.59	57.65
7	61.36	57.89	51.51	47.94	62.01	55.31	53.27	63.05
8	66.25	64.11	57.12	51.98	65.11	62.15	61.03	68.98
9	72.34	69.26	64.20	59.02	73.14	68.11	67.14	74.13
10	78.36	75.57	70.95	64.97	78.77	71.84	73.08	78.76
11	80.36	83.47	78.16	70.25	85.89	74.21	79.57	85.61
12	87.22	90.18	85.72	75.23	95.56	77.80	87.03	91.55

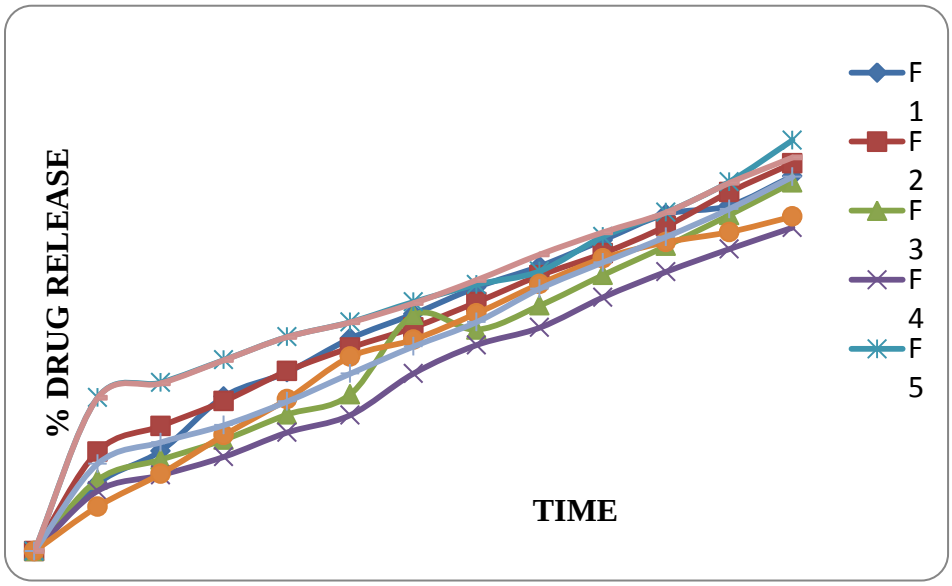

Figure 12: *In vitro* drug release profile of Acyclovir from F1 to F8

In vitro drug release studies of Acyclovir from floating microspheres were performed in pH 1.2 for12 hrs. In USP XX III dissolution test apparatus, it was found that *in vitro* drug release of formulation F1 to F3d containing ethyl-cellulose.F1, F2, F3, F4, F5, F6, F7, and F8 show percentage drug release 75.23% to 95.56% at end of 12 hour. Amongst the formulation F5 was

found to be the best formulation as it releases Acyclovir 95.56 % in a controlled manner with constant fashion up to 12hr. It was observed as the concentration of ethyl-cellulose was increased, percentage release of drug also increases.

The change of stirring speed also influenced the drug release from microspheres as shown in Figure 4. It was observed that the amount of drug release increases at the lower speed .This may be due to the adherence of drug particles on the surface of polymeric matrices .Whereas, at higher speed drug release further decreases .However the best release was observed with formulation F5, at the stirring speed of 800 rpm. Lower volume of processing medium shows higher amount of drug release as compared to higher volume of processing medium as shown in Figure 4. The best release with the formulation F5 when the volume of processing medium was 90 ml.

Table 5: Drug Release Kinetics for Best Formulation (F5)

S.No	Time (hours)	Square root of time	Log time	Cum %drug release	Log cum % drug release	Cum % drug remaining	Log cum % drug remaining
1	1	0.707	0.000	35.69	1.544	65	1.813
2	2	1.414	0.301	39.21	1.591	61	1.785
3	3	1.732	0.477	44.46	1.643	56	1.748
4	4	2.000	0.602	49.82	1.690	51	1.707
5	5	2.236	0.699	53.27	1.724	47	1.672
6	6	2.449	0.778	57.96	1.756	43	1.633
7	7	2.646	0.845	62.01	1.792	38	1.579
8	8	2.828	0.903	65.11	1.813	35	1.544
9	9	3.000	0.954	73.14	1.863	27	1.431
10	10	3.162	1.000	78.77	1.892	22	1.342
11	11	3.317	1.041	85.89	1.929	15	1.176
12	12	3.464	1.079	95.56	1.978	5	0.698

Table 6: kinetic Studies (F5)

	Zero order	First order	Higuchi modal	Peppas modal
Slope	6.8462	0.0196	23.5864	0.6824
r2	0.982	0.901	0.983	0.996

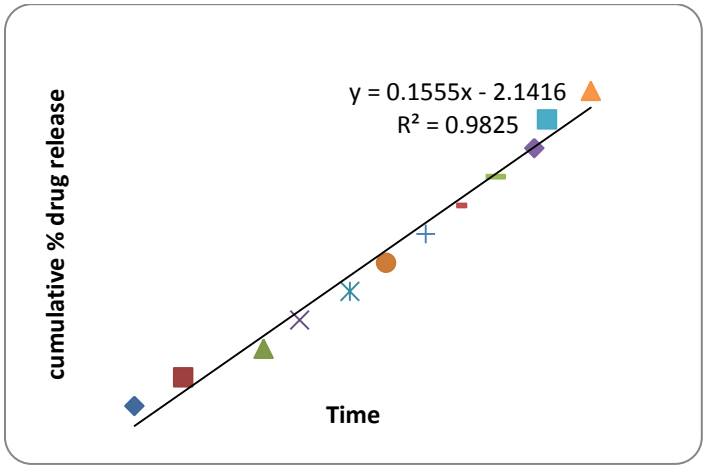

Figure 13: Zero order kinetics

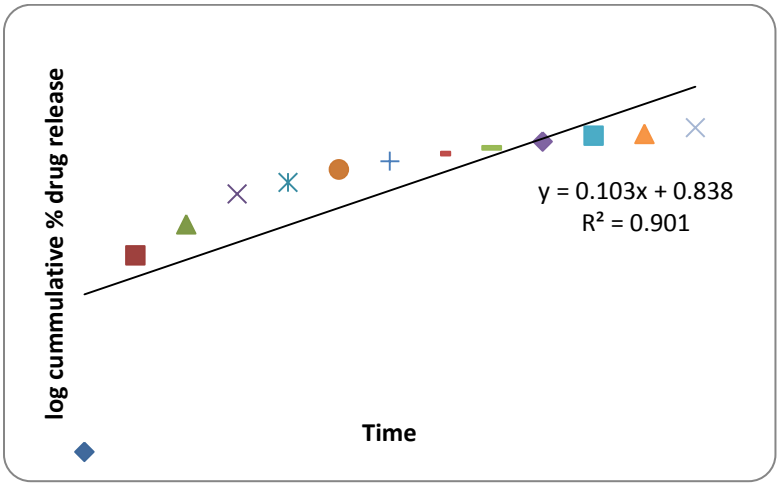

Figure 14: First order kinetics

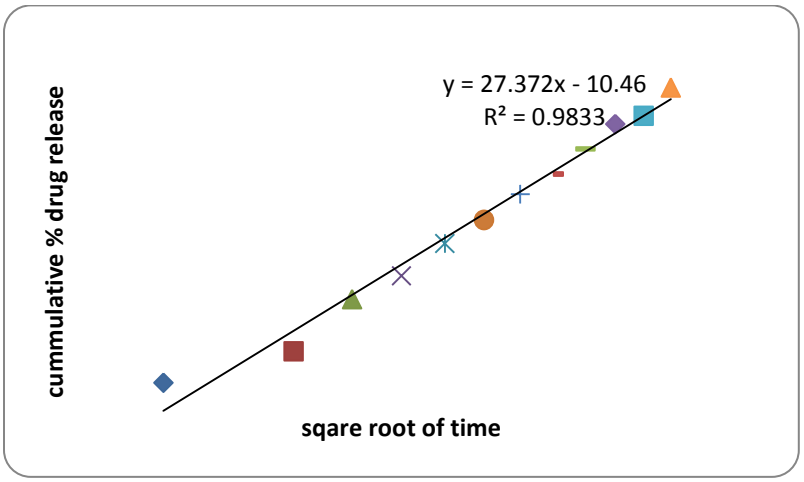

Figure 15: Higuchi model

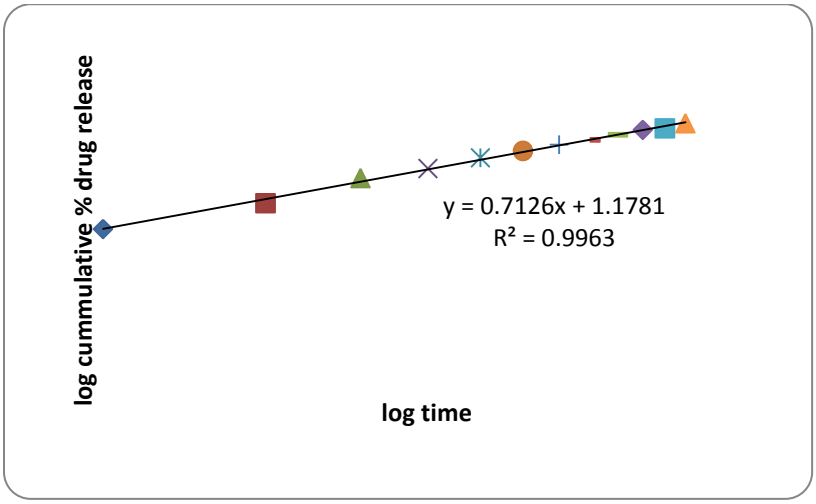

Figure 16: Korsemeyer Peppas model

The results of dissolution data fitted to various drug release kinetic equations. All the formulations followed zero order release due to its higher correlation coefficient values. The mechanism of drug release was non fickian. The kinetic values obtained for different formulations are tabulated in Table 6.

Table 7: Accelerated stability studies data of best formulation (F5)

S. No	Test	Initial	Period in months (40^0C / 75 %RH)		
			1	2	3
1	Physical appearance	white	white	white	white
2	Particle size(µm)	317.8±2.12	316.8±1.40	316.1±2.08	315.4±1.14
3	Incorporation efficiency (%)	88.4±0.46	87.9±0.1 2	87.2±0.97	87.2±0.10
4	Percentage yield (%)	87.0±0.56	87.5±0.46	87.3±0.32	86.3±0.41
5	In-vitro Buoyancy (%)	91.89±1.91	91.54±1.6	90.53±0.94	91.0±0.87
6	*In-vitro* drug Release (%) (12 hour)	95.36	95.24	95.05	95.03

The selected Formulation F5 was evaluated for stability studies. Microspheres were stored in High density polyethylene container at 40^0C / 75 % RH for 3 months.

i) Particle size: No significant change was observed in the particle size after storage period of 3 months at 40^0C / 75 % RH condition

ii) In vitro Buoyancy: No significant change was observed in the In-vitro buoyancy test after storage of 3 months. It was found to be 91%.

iii) Dissolution: No significant change was observed in the percentage drug dissolved after storage period of 3 months at 40^0C / 75 % RH condition for microspheres of acyclovir. The drug release was 87.9% at the end of 12 hrs.

iv) Assay: No significant change was observed in the assay value after storage period of 3 months at 40^0C / 75 % RH condition for microspheres of acyclovir. The drug content was found

to be 86.3 %.

CONCLUSION

The *in vitro* evaluation study of Acyclovir loaded floating microspheres was greatly improved when compared with those of conventional dosage form. Among all formulations, F5 was found to be satisfactory in terms of excellent micrometric properties, percentage yield, percentage drug entrapment efficiency, in vitro buoyancy, and highest in vitro drug release of 95.56% in a sustained manner with constant fashion over extended period of time. All the formulations followed zero order release. The mechanism of drug release was non fickian. Thus the study fulfilled the objective of developing efficient acyclovir microspheres.Hence, finally it was concluded that the prepared floating microspheres of Acyclovir may prove to be potential candidate for safe and effective controlled drug delivery over an extended period of time which can reduce dosing frequency.

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