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FORMULATION AND EVALUATION OF GASTRORETENTIVE FLOATING HOLLOW MICROSPHERE (MICROBALLON) LOADED WITH ACYCLOVIR

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ABSTRACT

The present study deals with the formulation of gastroretentive hollow microsphere of Acyclovir using Eudragit RS 100 & Ethyl cellulose as a polymer & solvent (Ethanol & Di chloro methane) in different ratio of solvent evaporation method. A number of evaluation parameter was carried out. A drug – exicipient compatibility studies was performed by FTIR. Microspheres were evaluated for particle size, micromeritic properties, flow property, drug entrapment efficiency, in vitro drug release , kinetic studies. The shape & size of microsphere were carried by Scanning Electron Microscopy.

KEYWORDS: **Acyclovir, Eudragit, Ethyl cellulose, microsphere**

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INTRODUCTION

One of the most feasible approaches for achieving a prolonged and predictable drug delivery profile in the GI tract is to control the gastric residence time i.e. Gastro retentive Dosage Forms (GRDFs) These are primarily controlled release drug delivery systems, which gets retained in the stomach for longer periods of time, thus helping in absorption of drug for the intended duration of time. Gastric retentive drug delivery devices can be useful for the spatial and temporal delivery of many drugs. Thus, control of placement of a DDS in a specific region of the GI tract offers numerous advantages, especially for drug exhibiting an 'absorption window' in the GI tract. The intimate contact of the DDS with the absorbing membrane and also the potential to maximize drug absorption may influence the rate of drug absorption. These considerations have led to the development of oral controlled release (CR) dosage forms possessing gastric retention capabilities. Drug may not be absorbed uniformly over the length of the gastrointestinal tract, because dosage form may be rapidly transported from more absorptive upper regions of the intestine to lower regions where the drug is less absorbed and drug absorption from colon is usually erratic and inefficient. Moreover, certain drugs are absorbed only from the stomach or the upper part of small intestine.The retention of oral dosage forms in the upper GIT causes prolonged contact time of drug with the GI mucosa, leading to higher bioavailability, and hence therapeutic efficacy, reduced time intervals for drug administration, potentially reduced dose size and thus improved patient compliance. Therefore, extended release DDS possessing gastric retention properties may be potentially useful [1].Hollow Microspheres are gastro-retentive drug delivery systems based on non-effervescent approach. Hollow Microspheres are in strict sense, spherical empty particles without core. These Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, ideally having a size 100 to1000 micrometer. Solid biodegradable Microspheres incorporating a drug dispersed or dissolved throughout particle matrix have the potential for controlled release of drugs. Gastro-retentive floating Microspheres are low-density systems that have sufficient buoyancy to float over gastric contents and remain in stomach for prolonged period. As the system floats over gastric contents, the drug is released slowly at desired rate resulting in increased gastric retention with reduced fluctuations in plasma drug concentration[2]. When Microspheres come in contact with gastric fluid the gel formers, polysaccharides, and polymers hydrate to form a colloidal gel barrier that controls the rate of fluid penetration into the device and consequent drug release. Acyclovir, BCS class III/ IV drug , is widely used in the treatment of Herpes simplex virus infection as well as vericella zoster infection. ACV is a guanosine analogue antiviral drug. It is one of the most commonly used antiviral drugs, that is primarily used for the treatment of herpes simplex virus infections, as well as in the treatment of varicella zoster (chickenpox) and herpes zoster (shingles) It.s 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. EC is a derivative of cellulose in which some of the hydroxyl groups on the repeating glucose units are converted into ethyl ether groups. The number of ethyl groups can vary depending on the manufacturer.It is mainly used as a thin-film coaterial.EC is currently used in pharmaceutical applications for micro encapsulation of actives, controlled-release matrix systems, taste masking, solvent and extrusion granulation, tablet binding, and as a controlled-release coating for tablets and beads.Apart from that, Ethylcellulose can function as an emulsifier to stabilize water-oil-mixtures.It functions as a binding and filling agent or serves as protective coating. Ethylcellulose is a water insoluble, at any pH, hydrophobic coating material[3] and due to its neutral side chains, it releases the drug in a pH-independent manner. It is often used for controlled release, taste masking It is non-toxic,

non-allergenic, non-irritant and widely used in oral drug delivery devices as polymeric film former.

MATERIALS AND METHODS

Acyclovir was obtained as a gift sample by my college IIMT College of Medical Sciences Meerut. Eudragit RS 100,Ethyl cellulose ,Ethanol & Di chloro Methane were supplied by Central drug House Ltd.

Preformulation Studies

Solublity Studies

Solublity of Acyclovir in different solvent was carried out & it reveals that Acyclovir is freely soluble in dimethylsulfoxide.

Melting Point Determination

Melting point of Acyclovir was determined by open capillary method.

Table 1
Melting Point

S.NO	Actual Melting Point	Observed Melting Point
1	256.5°C (494°F)	250.5°C(482.9°F)

Drug Polymer Interaction (FTIR) Studies

From the spectra of Acyclovir, mixture of Acyclovir & polymer was observed that all characteristic peaks of Acyclovir were present in combination specta ,thus indicating compatibility of Acyclovir & polymer.

Table 2
Reported Frequencies Range

Functional Groups	Frequencies Range (cm^{-1})	Observed Frequencies (cm^{-1})
N-H	3500-3310	3437.53
-OH	3550-3450	3437.00
C=O	1725-1680	1632.43
C-C	1600-1450	1542.02
N-O	1370-1300	1387.11
C-N	1340-1250	1217.24

Standard calibration curve of Acyclovir drug

Standard calibration curve was prepared by using Shimadzu (1800) UV Spectrophotometer at 254nm.

Preparation of Hollow Microsphere of Acyclovir

Hollow microspheres containing Acyclovir drug in their outer polymeric shell were prepared by emulsion solvent Evaporation method. Weighed amount of Acyclovir was mixed with polymers (Eudragit-S 100 and Ethyl Cellulose) in different ratios in a mixture of Dichloromethane and Ethanol (1:1) at room temperature. A vortex homogenizer was used for make a homogenous mixer of drug and polymer. This solution was added drop wise to light liquid paraffin containing 0.5% Tween 80 as an emulsifying agent. The beaker and its content were heated at 40°c with constant stirring 1000 rpm for 1.5 hours using using a three blade propeller stirrer to form a w/o emulsion. After complete evaporation of aqueous phase the liquid paraffin was decanted and collected microspheres were washed three times with n-hexane to remove liquid paraffin. The microspheres were dried and stored in vacuum desiccators.

Table 3
Formulation Table

Formulation	Acyclovir(gm)	Ethyl Cellulose (gm)	Eudragit (gm)	Ethanol:DCM(ml)
F_1	0.2	0.1	0.0	1:1
F_2	0.2	0.1	0.1	1:1
F_3	0.2	0.1	0.2	1:1
F_4	0.2	0.2	0.1	1:1
F_5	0.2	0.2	0.2	1:1
F_6	0.2	0.2	0.3	1:1

Evaluation of Acyclovir Microsphere

1. Percentage yield[4]

The percentage yield of the floating microspheres was determined for drug and was calculated using the following equation

% Yield = (Actual weight of product / TotalWeight of excipient and drug) × 100 ------ (1)

2. Particle size

Calibrate the eye piece micrometer and transfer the floating microspheres on clean slide. Add one or two drops of liquid paraffin. Disperse the sample uniformly with the help of a brush. Place the cover slip to avoid entrapment of air bubbles. Drain the excess liquid with a blotting paper. Place the slide on the stage of the microscope. Focus the slide in low magnification (10X). Observe the presence of individual particle. Shift to high power (45X) and focus the slide. Measure the size of each particle in terms of eye-piece divisions. Tabulate the particle in terms of divisions of eye-piece and number of particles. Multiply the number of eye-piece divisions by the calibrated value. Classify the diameters into size ranges and calculate the number of distribution.

3. Bulk density

It is the ratio of mass of the blend to bulk volume. It was measured by pouring powder in measuring cylinder and measuring the volume occupied by powder.

4. Tapped density[5]

It is the ratio of mass of the blend to tapped volume. It was measured by digital tapdensitometer by measuring the volume occupied by powder after 100 standard tapping.

5. Carr's (compressibility) index

Compressibility index of micro particles was computed according to the following equation

% compressibility = Tapped density – Bulk density / Tapped density X 100------(2)

The value given below 15% indicates a powder with usually give rise to good flow characteristics, whereas above 25% indicate poor flow ability.

6. Hausner's ratio

Hausner's ratio of microspheres was determined by comparing tapped density to bulk density Using equation

$$\text{Hausners ratio} = \frac{\text{Bulk density}}{\text{Tapped density}} \quad \text{- - - - - - (3)}$$

Values less than 1.25 indicate good flow (= 20% Carr), whereas greater than 1.25 indicates poor flow (= 33% Carr).

7. Angle of repose

Angle of repose (q) of the microspheres was measured using funnel method. The microspheres was poured through a funnel that can be raised vertically until a maximum cone of height was obtained. The radius of heap was measured and angle of repose was calculated.

tan θ = h/r- - - - - - -(4)

Where, θ = Angle of repose, h = Height of granules above the flat surface, r = radius of the circle formed by the granule heap.

8. Scanning electron microscopy[6]

Dry microspheres are placed on an electron microscope brass stub a coated with gold in an ion sputter. Then picture of microsphere were taken by spectro random scanning of the stub. The microspheres are viewed at an accelerating voltage of 20KV.

9. IR spectroscopy[7]

The Fourier transform infra-red analysis was conducted for the analysis of drug polymer interaction and stability of drug during microencapsulation process.

10. Percentage Yield.[8]

The prepared microspheres were collected and 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.

% Yield = (Actual weight of product / total Weight of excipient and drug) × 100 - - - - **(5)**

11. DEE (Drug Entrapment Efficiency) [9]

The various formulations of the floating microspheres were subjected for drug content. 50 mg of floating microspheres from all batches were accurately weighed and crushed. The powdered of microspheres were dissolved with 10ml di methyl sulfoxide in 100ml volumetric flask and makeup the volume with 0.1 N HCl. This resulting solution is than filtered through whattman filter paper No. 44. After 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 HCl and the absorbance was measured at 233 nm against 0.1 N HCl as a blank. The percentage drug entrapment was calculated as follows.

DEE = (Amount of drug actually present/Theoretical drug load expected) × 100 - - - - (6)

12. Floating behavior of Floating microsphere[10]

100 mg of the floating microsphere were placed in 0.1 N HCl (300 ml) containing 0.02% of tween 20. The mixture was stirred with paddle at 100rpm. The layer of buoyant microspheres was pipette and separated by filtration at 1, 2, 4 and 6 hours. The collected microspheres were dried in desiccators over night. The percentage of microspheres was calculated by the following equation

%Floating microspheres= (weight of floating microspheres/ initial weight of floating microspheres) × 100 - - - - - - (7)

13 In-vitro Release Studies[11]

The drug release rate from floating microspheres was carried out using the USP type II (Electro Lab.) dissolution paddle assembly 38. A weighed amount of floating microspheres equivalent to 100 mg drug were dispersed in 900 ml of 0.1 N HCl (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.

14 Stability Studies

The stability studies of the finalized formulations were designed and carried out as per ICH guidelines. The optimized formulations filled in capsule were stored in screw capped glass container covered with aluminum foil in order to minimize the accidental exposure of the sample to the light. The formulation was stored at 27±5°c /60±5% RH and 40º ± 2ºC/60% ± 5% RH for 3months. After intervals of 0, 30, 45 and 90 days, samples were withdrawn and retested for drug release.

15. Kinetics of Drug Release

To examine drug release kinetics and mechanism cumulative release data were fitted to model representating Zero order(Q v/s T) , First order [Log(Q_0-Q)v/s T], Higuchi Model(Qv/sT½), Peppas model and Hixson model.

RESULTS & DISCUSSION

i)Preformulation Studies

a)Standard Calibration Curve of Acyclovir in Acidic Buffer (λ_{max} =254nm)

Table 4
Absorbance at pH 1.2

Concentration(µg/ml)	Absorbance at 254 nm
10	0.024
20	0.056
30	0.076
40	0.089
50	0.124
60	0.154
70	0.182
80	0.197
90	0.215
100	0.234

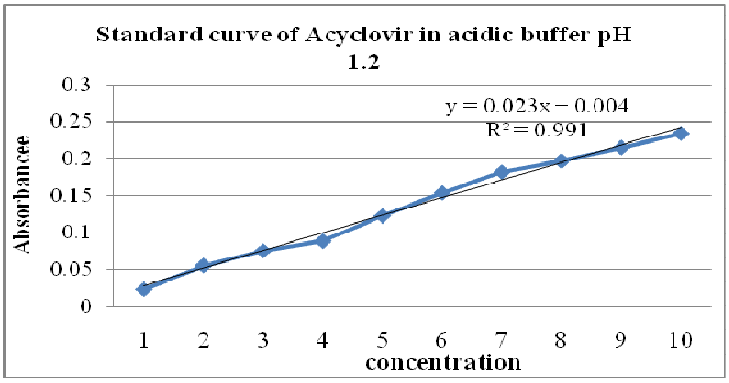

Figure 1
Plot for Calibration curve in acidic buffer

b) Drug Polymer Interaction (FTIR) Studies

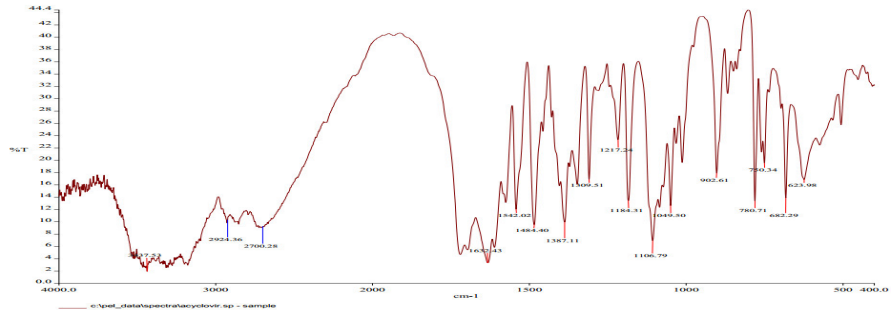

Figure 2
FTIR Spectrum of Acyclovir

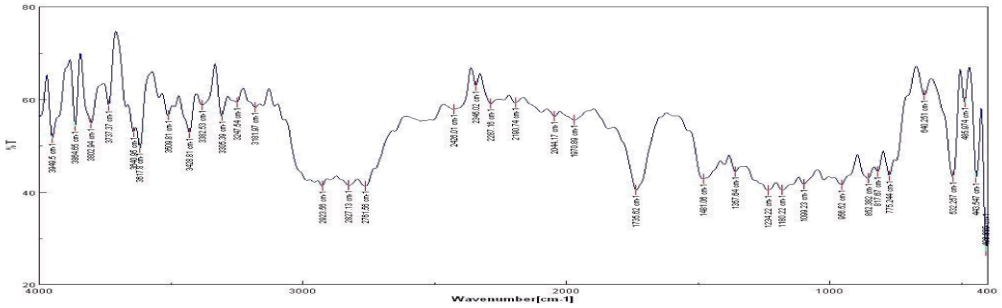

Figure 3
FTIR of Eudragit RS 100

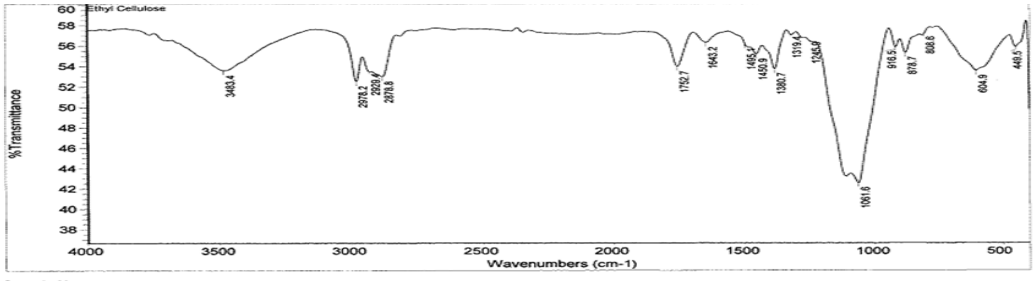

Figure 4
FTIR of Ethyl Cellulose

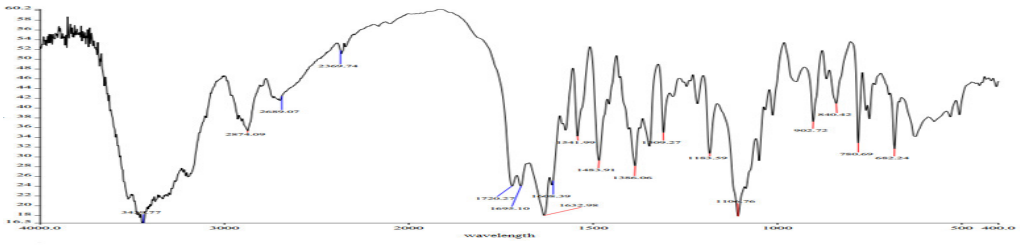

Figure 5
FTIR of Acyclovir + Polymer

Ii) Evaluation Parameter

1) Particle size

Table 5
Particle size

Formulation	Mean Particle Size (µm) SD±n=3
F1	340±2.2
F2	386±2.7
F3	427±3.5
F4	440±3.9
F5	478±2.5
F6	505±3.1

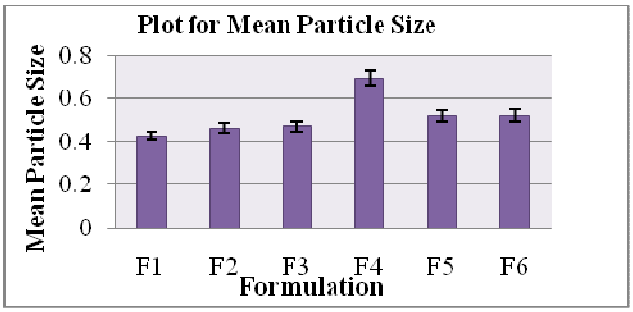

Figure 6
Plot for Mean Particle Size

2) Micrometric Properties

Table 6
Tapped Density

Formulation	Tapped density (gm/cm^3) S D±(n=3)
F1	0.428±0.002
F2	0.464±0.008
F3	0.474±0.01
F4	0.695±0.021
F5	0.522±0.009
F6	0.524±0.012

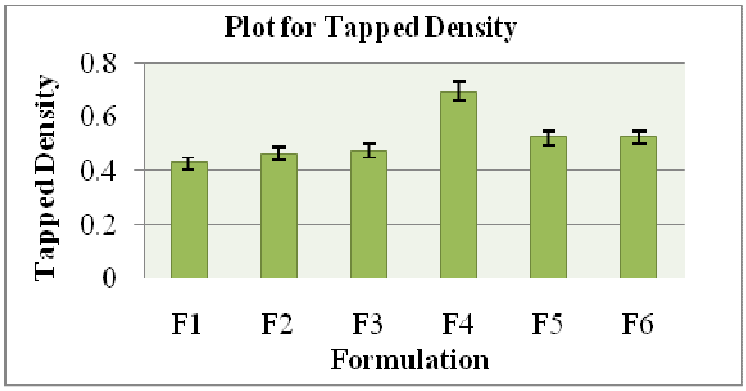

Figure 7
Plot for Tapped Density

Table7
Angle of Repose

Formulation	Angle of Repose(º)SD ± n=3
F1	17.91±0.42
F2	19.66±0.20
F3	19.81±0.54
F4	25.01±47
F5	22.64±52
F6	24.16±63

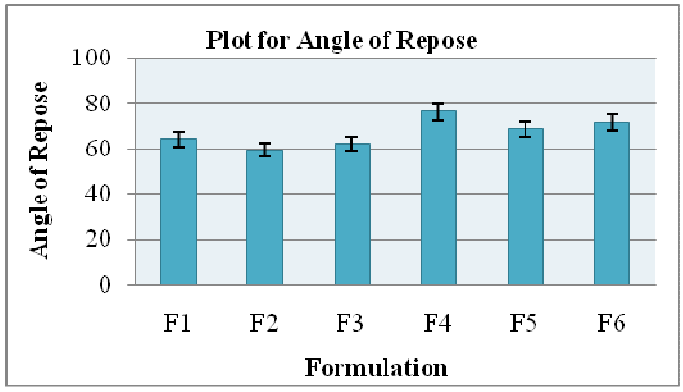

Figure 8
Plot for Angle of Repose

Table 8
Carr's Index

Formulation	Carr's Index SD± n=3
F1	15.65±0.696
F2	17.02±0.432
F3	17.93±1.465
F4	16.75±0.765
F5	13.79±1.231
F6	17.01±0.742

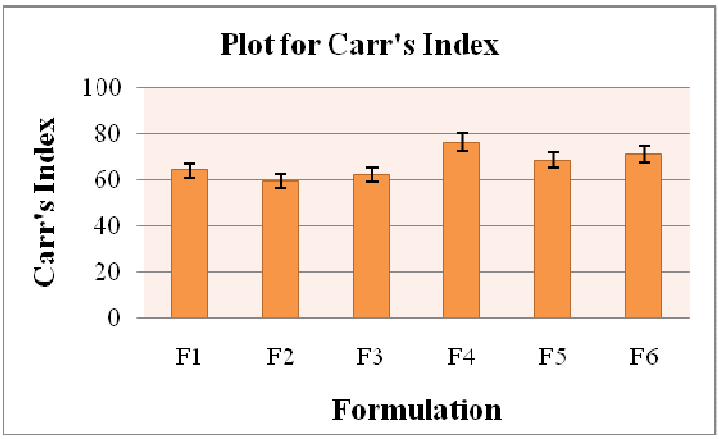

Figure 9
Plot for Carr's Index

3) Percentage Yield

Table 9
Percentage Yield

Formulation	Percentage Yield(%) SD± n=3
F1	76.49±0.011
F2	78.10±0.015
F3	75.23±0.015
F4	82.00±0.011
F5	80.66±0.015
F6	78.34±0.012

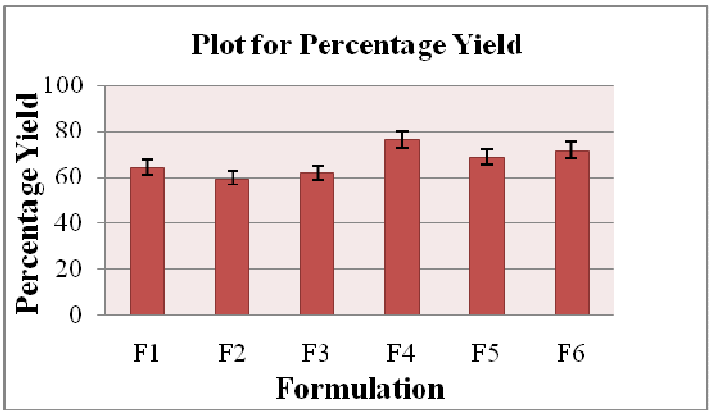

Figure 10
Plot for Percentage Yield

4) Drug Entrapment Efficiency

Table 10
Drug Entrapment Efficiency

Formulation	DEE(%w/w) SD± n=3
F1	64.10±1.7
F2	59.46±1.3
F3	62.00±1.5
F4	76.23±3.1
F5	68.55±2.5
F6	71.40±2.9

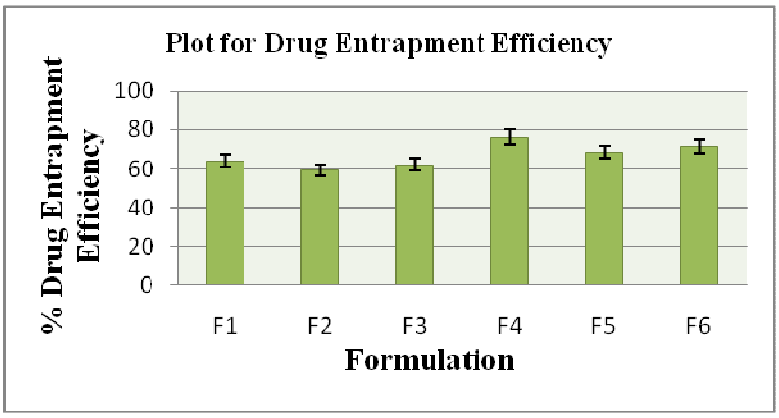

Figure 11
Plot for DEE

5) Floating behavior

Table 11
Floating Behavior of Formulation

Formulation	1 hr SD ± n=3	2hr SD± n=3	4hr SD ±n=3	6hr SD± n=3
F1	78.1±0.49	77.8±0.47	75.4±0.26	72.2±0.112
F2	74.34±0.28	73.15±0.34	71.22±0.45	70.89±0.128
F3	71.15±0.39	69.23±0.43	66.55±0.31	65.50±0.196
F4	85.12±0.27	83.00±0.44	82.56±0.18	81.67±0.015
F5	82.11±0.45	81.45±0.38	79.89±0.86	78.55±0.105
F6	78.76±0.33	76.50±0.21	75.78±0.060	71.21±0.065

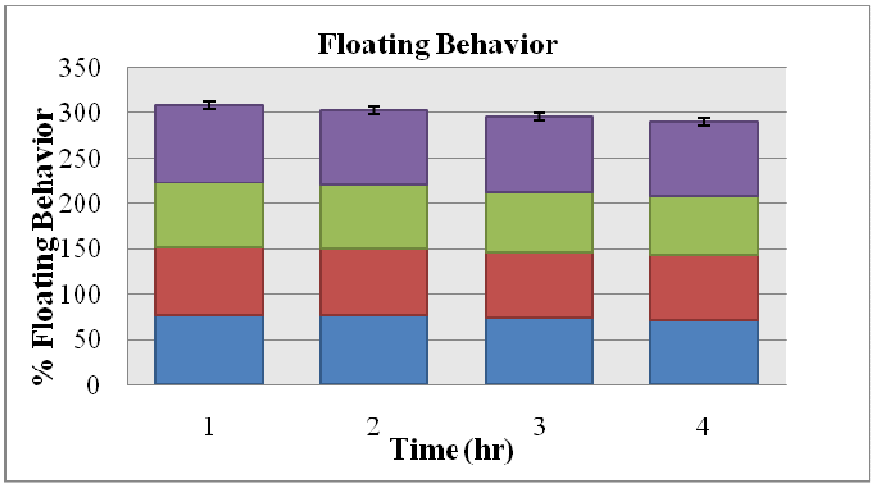

Figure 12
Plot for Floating Behaviour

6) Scanning electron microscopy

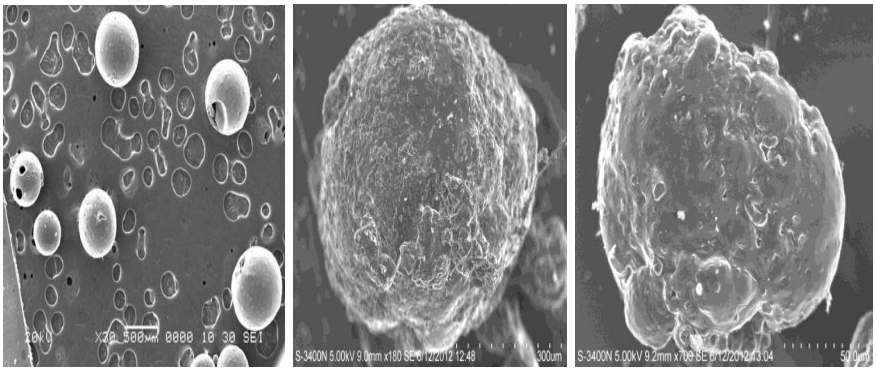

Figure 13
a)Microsphere loaded with Acyclovir (b) Single Microsphere before dissolution (c) Microsphere having porous surface

7) In Vitro Drug Release

Table 12
Table for in vitro drug release studies

S.NO	Time(hr)	Percentage	Cumulative	drug	release		
		F1	F2	F3	F4	F5	F6
1	1	18.81±0.62	19.97±0.58	19.23±0.34	17.47±0.09	17.68±0.21	19.33±0.28
2	2	27.65±0.47	28.53±0.49	28.30±0.41	28.45±0.58	25.98±0.37	25.86±0.47
3	3	32.44±0.67	32.53±0.56	34.30±0.28	34.52±0.28	37.48±0.49	36.66±0.58
4	4	46.12±0.54	42.49±0.57	43.48±0.63	43.24±0.32	45.61±0.62	45.39±0.67
5	5	52.45±0.42	54.62±0.39	54.74±0.26	56.75±0.36	56.34±0.38	55.49±0.63
6	6	66.89±0.52	66.67±0.65	67.1±0.19	68.67±0.59	69.19±0.60	60.59±0.49
7	7	75.87±0.63	78.56±0.31	78.45±0.59	77.41±0.67	77.61±0.59	78.95±0.51
8	8	82.24±0.45	80.21±0.29	81.44±0.40	81.56±0.65	80.54±0.39	80.75±0.59
9	9	87.34±0.48	85.89±0.56	83.45±0.61	83.7±0.54	84.56±0.42	86.54±0.47
10	10	91.20±0.34	89.28±0.65	90.23±0.33	89.54±0.41	90.11±0.35	91.31±0.68
11	11	96.86±0.78	92.56±0.54	91.67±0.56	93.89±0.22	93.18±0.53	92.57±0.40
12	12	98.69±0.65	97.47±0.68	98.86±0.21	99.28±0.45	97.45±0.66	98.27±0.56

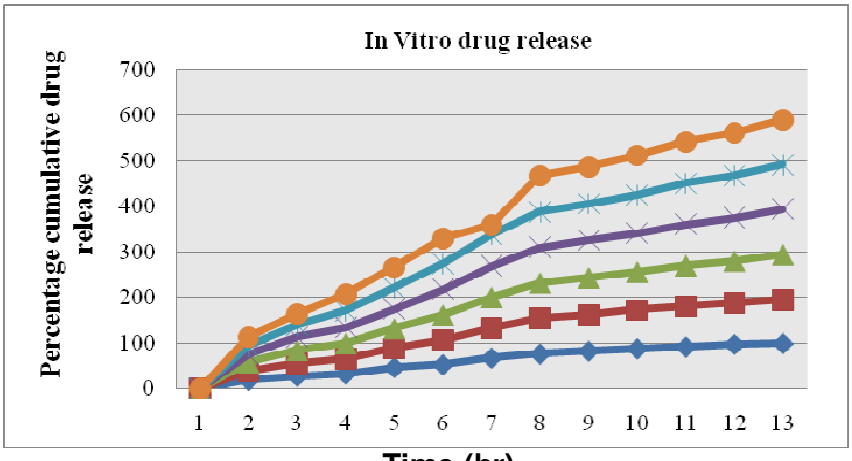

Figure 14
Plot for In Vitro Drug Release

8) Stability Studies

Table 13
Stability Studies of formulation F4

S. No	Days	%drug remaining 27±2^{0}c/60±5% RH	%drug remaining 42±2^{0}c/60±5% RH
1	0	100±0	100±0
2	30	99.9±.003	99.4±.041
3	45	98.8±.027	98.2±.036
4	90	97.6±.012	97.1±.02

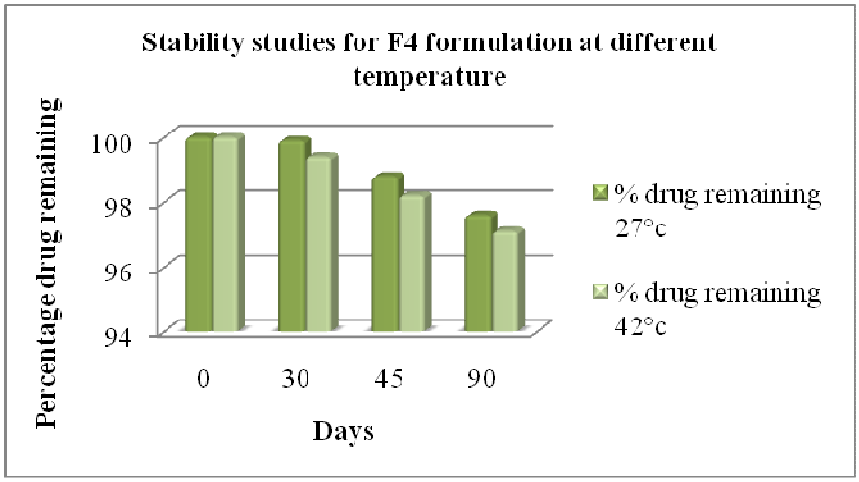

Figure 15
Plot for stability studies of F4 Formulation 9)Release Kinetics

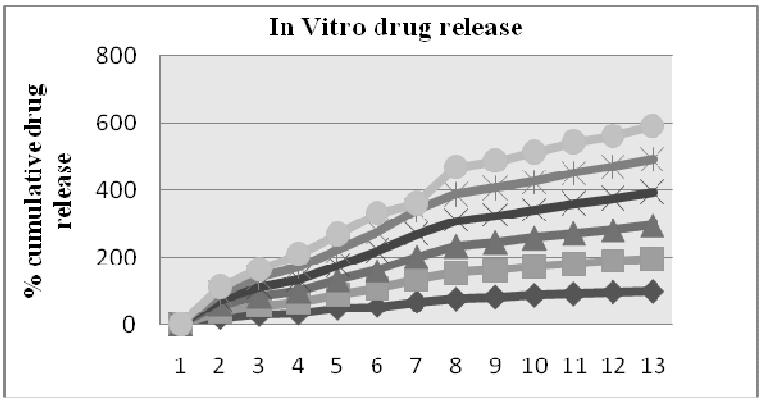

Figure 16
Plot for Zero order release

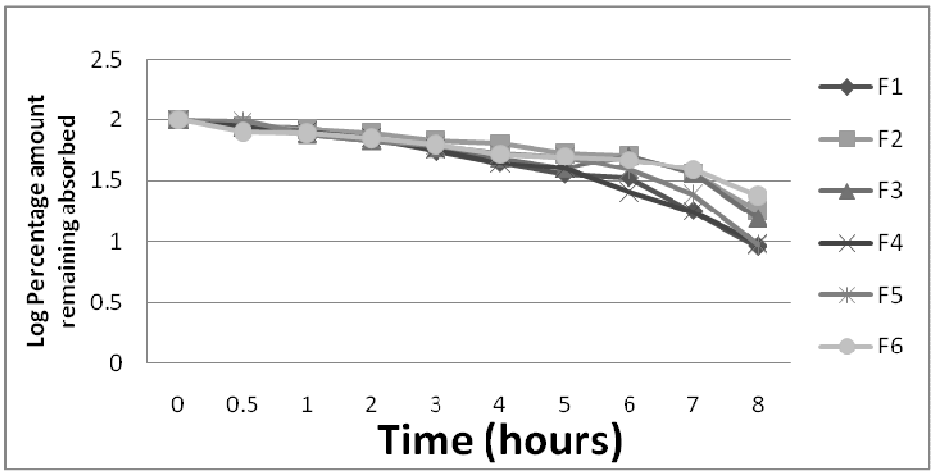

Figure 17
Plot for First order release

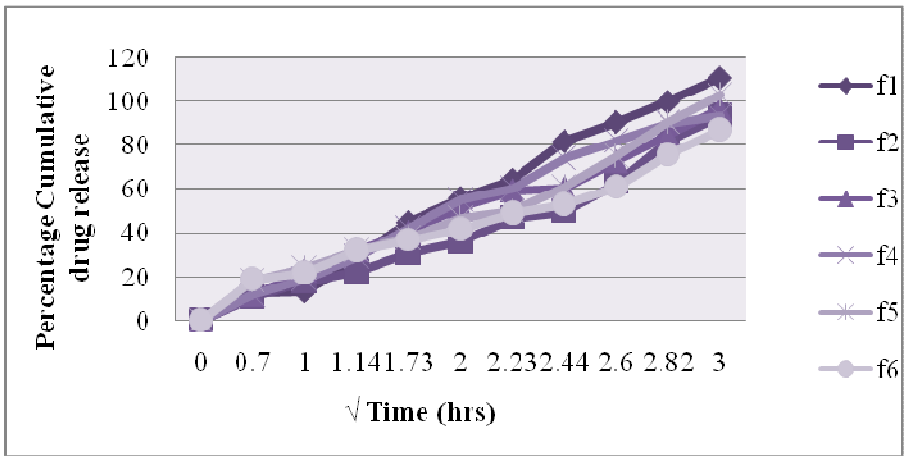

Figure 17
Plot for Higuchi Model

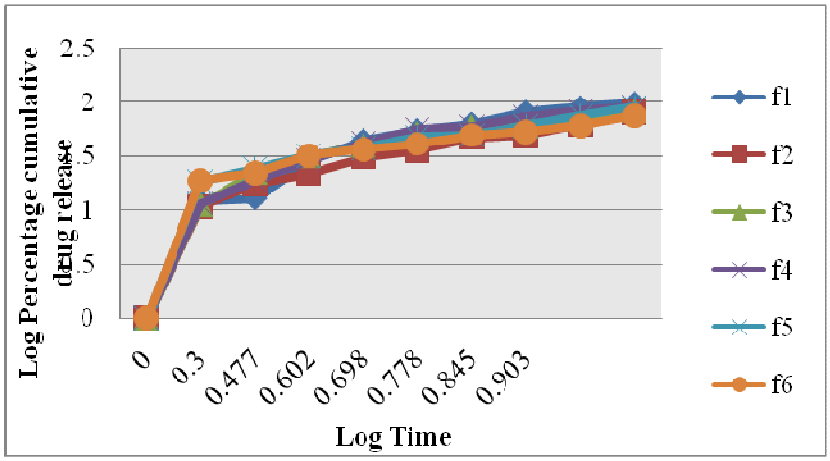

Figure 18
Plot for Peppas Model

DISCUSSION

The present study report a novel attempt to prepare microsphere of Acyclovir by using ethyl cellulose & eudragit as a polymer. Microsphere of acyclovir was prepared by solvent evaporation method & evaluation parameter were assessed with a view to obtain sustained release microsphere. In the present work total six formulations were prepared. The prepared microsphere were subjected to FTIR, SEM, Particle size analysis, % yield , drug entrapment efficiency, in vitro dissolution.

Preformulation Studies

Solublity of Acyclovir in different solvent was carried out & it reveals that Acyclovir is freely soluble in dimethylsulfoxide. Melting point of Acyclovir was found to be 250.5ºc which complied with IP standards thus indicating purity of drug sample. FTIR Spectrum indicate that there are no interaction between drug and polymer all the ingredient are compatible to each other. Standard calibration curve of ACV was observd at pH 1.2 by using UV Spectrophotometer at λ_{max} 254nm.

Evaluation of Acyclovir Microsphere

The SEM micrographs revealed that the resulting microspheres were spherical in nature with rough surfaces containing cracks and holes over its surface. The micrographs showed almost spherical but the morphology appeared to be rough . The reason behind this morphology change can be attributed to the faster evaporation of dichloromethane forming a pore like structure. The mean particle size of the microspheres significantly increased with increase in polymer concentration and was in the range of 275-340 µm .The percentage yield for Acylovir microsphere were 76.49%,78.10%, 75.23%, 82%,80.66% & 78.23% For formulation F1,F2,F3,F4,F5,& F6 respectively. Entrapment efficiency increases with increases polymer concentration. The % entrapment efficiency was found to be 61.10 to 81% w/w. By increasing polymer concentration drug entrapment efficiency increased.

FTIR spectra were obtained for Acyclovir, mixture of drug & polymer. The characteristic peaks of the drug were compared with the peaks obtained for physical mixture of drug & polymer. The characteristic peaks were found in acyclovir , physical mixture, hence it appears that there was not chemical interaction between acyclovir & polymer.In Vitro dissolution studies of Acyclovir microsphere showed prolonged & sustained release of Acyclovir. The *in vitro* drug release profiles of all the formulations have been shown in figure . The release of acyclovir mainly depends upon the polymer concentration. The release rate of the drug from the microspheres was found to decrease drastically with increase in polymer concentration. Acyclovir release from all the formulations was found to be slow and sustained over 12 h. Bye the end of 12 h, Formulations F1, F2, F3, F4, F5, and F6 were found to release 98.69%, 97.47%, 98.86%, 99.28%, 97.17%, and 99.97% respectively. The plot of Cumulative percentage drug release against Time, Cumulative percentage drug release against root time, LOG percentage Cumulative drug retained against Time and Log percentage cumulative drug release against Log time were drawn and represent graphically. The co-efficient of determination indicates that the release data was best fitted with Higuchi Model. Higuchi equation explains diffusion controlled release mechanism.The diffusion Exponent ‘n’values of Korsemeyer-Peppas model was found to be between 0.5-1 indicating Non Fickian microsphere.

CONCLUSION

Preformulation studies like solubility, melting point, & UV analysis of Acyclovir were compiled with IP standards. The FTIR spectra revealed that there was no interaction between polymer & Acyclovir. All the polymers used were compatible with drug**.** Surface Morphology of microsphere was indicated that microsphere was spherical in shape due to less amount of eudragit polymer. If conc. of eudragit increased so microsphere can be burst**.** The SEM micrographs revealed that the

resulting microspheres were spherical in nature with rough surfaces containing cracks and holes over its surface. The mean particle size of the microspheres significantly increased with increase in polymer concentration. Entrapment efficiency increases with increases polymer concentration. From the result, it was revealed that there was a proper distribution of Acyclovir in the microspheres. The study also indicated that the release rate of the drug from the microspheres was found to decrease drastically with an increase in polymer concentration. In Vitro dissolution studies of Acyclovir microsphere showed prolonged & sustained release of Acyclovir. The co-efficient of determination indicate that release data was best fitted to Higuchi model .Regression co-efficient value in Korsemeyer Peppas Model was found to be 0.5-0.7 for microsphere of Acyclovir indicating Non Fickian microsphere. From the study it is evident that promising gasrtoretentive microsphere of ACV was prepared by solvent evaporation technique using ethyl cellulose & eudragit RS100 as polymer. Stability study indicates that microsphere are stable at different temperature. There are no changes in formulation F4 so it is a stable formulation of Acyclovir microsphere prepared by solvent evaporation method using ethyl cellulose and eudragit RS 100 as polymer.

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