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Preparation of Floatingmicrospheres of Acyclovir by Emulsion Solvent Diffusion Technique:

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ABSTRACT: The aim of work was to improve the oral bioavailability of the poorly water soluble drug by incorporating in floating drug delivery system. For better absorption and enhanced bioavailability of some drug, prolongation of retention time of the dosage form in the stomach is essential. In the present study Acyclovir was selected as model drug as it is the prototype antiviral agent used to treat various types of herpes infections having short half-life (2.5-3.3 hours) and low bioavailability (15-30%) in the upper part of GIT hence, it is suitable for gastro-retentive system. Ethyl cellulose was used to achieve the controlled delivery of drug from polymer matrix and emulsion solvent diffusion techniqueis selected for formulation. The particle size of floating microspheres shows different size for different formulation; this may due to variation in the composition of formulations. The mean particle size for all formulations was in the range of 135.103 – 229.418µm.

Keywords: Gastric residence time (GRT),Hydrodynamically balanced system (HBS),Acyclovir, Ethyl cellulose,Gastric emptying time (GET),Microspheres.

I. INTRODUCTION:

Oral drug delivery has been known for decades as the most widely used route of administration among all the routes. Oral delivery of drugs is the most preferable route of drug delivery due to ease of administration, patient compliance and flexibility in formulation. Pharmaceutical product designed for oral delivery which are currently available in the market mostly immediate-release or conventional release, which maintains the drug concentration within the therapeutically effective range only, when administered several times a day.[1]

The design of an oral controlled drug delivery system (DDS) should be primarily aimed at achieving more predictable and increased bioavailability of drugs. Several difficulties are faced in designing controlled release system for better absorption and enhanced bioavailability .Various approaches have been made to prolong the retention time of dosage form in the stomach. Retention of drug delivery system with prolonged overall gastrointestinal transit time and slow but completerelease in the stomach improves bioavailability of drugs that have site specific absorption from stomach.[2]

Furthermore, the relatively brief gastric emptying time (GET) in humans, which normally averages 2-3 hours through the major absorption zone (stomach or upper part of the intestine), can result in incomplete release from the drug delivery system (DDS) leading to decreased efficacy of the administered . Thus, control of placement of a DDS in a specific region of the gastrointestinal (GI) tract offer numerous advantages, especially for drugs exhibiting an absorption window in the GI tract or drugs a stability problem. Overall, the intimate contact of the DDS with the absorbing membrane has the potential to maximize drug absorption and may also influence the rate of drug absorption. These considerations have been tried to increase residence time and prolong drug release. One such method is the preparation of a device that remains buoyant in the stomach contents due to its lower density than that of the gastric fluids.[3-6]

The gastric emptying of a multiparticulate floating system would occur in a consistent manner with small individual variation. On each subsequent gastric emptying, sink particles will spread out more uniformally over a large area of absorption sites, increasing the opportunity for drug release profile and absorption in a more or less predictable way. Moreover, since each dose consists of many subunits the risk of dose dumping is reduced.[7-8]

Floating 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

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polymers, ideally having a size less than 200 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.[9]

II. MATERIALS AND METHODS:

Materials-

Acyclovir,Ethyl cellulose,Triethyl Citratewas obtained as kind gift sample fromWockhardt Pvt. Ltd, Aurangabad. Dichloromethane & Conc. HCL were purchased from Research Lab Ltd, Poona. Polyvinyl alcohol received form Qualigens fine chemicals, Mumbai.& Tween 20 received from Loba Chemie Pvt, Ltd, Mumbai.

Method:

Preparation of FloatingMicrospheres of Acyclovir by Emulsion Solvent Diffusion Technique

Floating microspheres containing acyclovir were prepared using emulsion solvent diffusion technique. For the preparation of floating microspheres, the rate controlling polymer used was ethyl cellulose of different viscosities (50cps and 100cps) in varying concentration (Drug: polymer, 1:1, 1:1.5 and 1:2). Triethyl citrate (TEC) was added as a plasticizer in this formulation in different concentration (10%and 20%).The drug and polymer mixture (1:1, 1:1.5 and 1:2) was dissolved in a dichloromethane (15ml) and plasticizer was added. The above mixture was dropped in a solution of polyvinyl alcohol (0.25%, 200 ml). The resultant solution was stirred with a mechanical stirrer for 1 hour at 500 rpm. The formed floating microspheres were filtered and washed with water and dried at room temperature and stored in a desiccator until further use. The various batches of floating microspheres were prepared as follows.[10]

Table No. 1Formulation of the floatingmicrospheres of acyclovir

Sr. No	Formulation code	Drug (Acyclovir) (gm)	Polymer Ethyl Cellulose (gm)		Plasticizer (TEC) (%)
			50 cps	100 cps	
1	A1	1	1	-	10
2	A2	1	1	-	20
3	A3	1	1.5	-	10
4	A4	1	1.5	-	20
5	A5	1	2	-	10
6	A6	1	2	-	20
7	B1	1	-	1	10
8	B2	1	-	1	20
9	B3	1	-	1.5	10
10	B4	1	-	1.5	20
11	B5	1	-	2	10
12	B6	1	-	2	20

EVALUATION OF MICROSPHERES:

Particle size analysis:

Particle size analysis plays an important role in determining the release characteristics and floating property. The sizes of floating microspheres were measured by laser diffraction particle size analyzer. Firstly, 1gm offloating microspheres was floated in 200 ml of containing 0.02 % of Tween 20 in aqueous solution and stirred at 37 ± 0.5 °C. Second, particle size distribution was obtained when a laser light passed through the microspheres and then diffracted the intensity in an angular distribution. The data obtained were evaluated using volume distribution diameter (d)

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values of 10%, 50% and 90%. The mean particle size was then calculated.[11]

Percentage yield:

The percentage yield of different formulations was determined by weighing the floating microspheres after drying. The percentage yield was calculated as follows.[12]

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

Drug entrapment:

The various batches of the floating microspheres were subjected to estimation of drug content. The floating microspheres equivalent to 50 mg of acyclovir from all batches were accurately weighed and crushed. The powdered of microspheres were dissolved in ethanol (10 ml) in volumetric flask (100ml) and made the volume with 0.1 N HCl. This solution is then filtered through Whatmann filter paper No. 44. After filtration, from this solution accurate quantity (10 ml) was taken and diluted up to 100 ml with 0.1 N HCl. From this solution, accurate volume (2 ml) was pipette out and diluted up to 10 m1 with 0.1 N HCI and the absorbance was measured at 254 nm against 0.1 N HCI as a blank. The percentage drug entrapment was calculated as follows.[13]

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

Scanning electron microscopy:

From the formulated batches of floating microspheres, formulation (A3) and (B3) which showed an appropriate balance between the buoyancy and the percentage release were examined for surface morphology and shape using scanning electron microscope.Sample was fixed on carbon tape and fine gold sputtering was applied in a high vacuum evaporator. The acceleration voltage was set at 20KV during scanning. Microphotographs were taken on different magnification and higher magnification (600X) was used for surface morphology.[14-15]

Fourier transforms infra-red spectroscopy (FT-IR) analysis:

The Fourier transform infra-red analysis was conducted for the analysis of drug polymer interaction and stability of drug during microencapsulation process. Spectrum of pure acyclovir, Ethyl Cellulose and floating microspheres were recorded.[16]

Powder X-ray diffraction:

The powder X-ray diffraction pattern of acyclovir and polymer were obtained using Phillips X-ray diffractometer with a Ni-filtered CuKα-radiation at a scanning speed of 10^0/min at 2θ.[17]

Floating ability of microspheres:

Floating microspheres (50 mg) were placed in 0.1 N HCI (100 ml) containing 0.02% Tween 20. The mixture was stirred at 100 rpm in a magnetic stirrer. The layer of buoyant microspheres was pipetted and separated by filtration at 1, 2, 4 and 8 hours. The collected microspheres were dried in a desiccator overnight. The percentages of microspheres were calculated by the following equation.[5]

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

In-vitro release studies:

In-vitro release of acyclovir from floating microspheres was carried out using the USP dissolution test apparatus (Type-I). A weighed amount of floating micro spheres equivalent to 200 mg of drug were filled into a capsule and placed in the basket. Dissolution media used was 900 ml of 0.1 N HCI (pH 1.2) maintained at 37 ± 0.5°C and

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stirred at 100 rpm. At predetermined time intervals, 10 ml of sample was withdrawn and replaced with equal amount of 0.1 N HCI (pH 1.2). The collected samples were filtered and suitably diluted with 0.1 N HCI and analyzed spectrophotometrically at 254 nm to determine the amount of drug released in the dissolution medium.

Powder X-ray diffraction:

The powder X-ray diffraction pattern of acyclovir and polymer were obtained using Phillips X-ray diffractometer with a Ni-filtered CuKα-radiation at a scanning speed of 10^0/min at 2θ.

III. RESULTS AND DISCUSSION

Particle size analysis:

Smaller the microspheres,floating ability will be less and faster will be the release rate of drug from microspheres, While larger the size, floating ability will be more and sustained will be the release of drug.

Table No. 2 Particle size of different batches of floating microspheres

Sr.No	Formulation code	Mean particle size (µm)
1	A1	152.531 ±2.85
2	A2	150.579 ± 3.53
3	A3	135.103 ± 1.43
4	A4	147.763 ± 3.12
5	A5	152.873 ± 2.17
6	A6	152.828 ± 1.86
7	B1	152.103 ± 2.16
8	B2	152.977 ± 3.26
9	B3	148.113 ±2.43
10	B4	229.418 ± 1.24
11	B5	147.965 ± 1.37
12	B6	150.676 ± 2.13

Micromeritics properties:

Angle repose of floating microspheres was observed in range of 17°.83'- 26°.22' i.e less than 30 as shown in Table-3. All formulation showed good free floating nature. Hausner's ratio of microparticles was determined by comparing the tapped density to the bulk density. It was ranging from 1.1529-1.2185; i.e. all the formulation showed that they had good flow properties.The bulk density value of different microspheres ranged from 0.361 - 0.532 gm/cm^{3}The Tapped density value of microspheres ranged from 0.422 - 0.631 gm/cm.^{3}

Sr.No	Formulation code	Angle of Repose	Hausner's Ratio	Bulk Denity gm/cm^3	Tapped Density gm/cm^3
1	A1	17°.91' ± 0.42	1.1855 ± 0.023	0.361 ± 0.005	0.428 ± 0.002
2	A2	17°.83' ±0.61	1.1887 ± 0.018	0.355 ± 0.007	0.422 ± 0.004
3	A3	19°.66' ± 0.20	1.2051 ± 0.020	0.385 ± 0.006	0.464 ± 0.008
4	A4	19°.81' ± 0.54	1.2185 ± 0.016	0.389 ± 0.007	0.474 ± 0.010
5	A5	19°.25' ± 0.48	1.1682 ± 0.025	0.422 ± 0.008	0.493 ± 0.009
6	A6	20°.26' ± 0.32	1.1529 ± 0.032	0.425 ± 0.007	0.490 ± 0.010
7	B1	17°.98' ± 0.61	1.1588 ± 0.028	0.447 ± 0.009	0.518 ± 0.013
8	B2	22°.64' ± 0.52	1.16 ± 0.042	0.450 ± 0.006	0.522 ± 0.009
9	B3	20°.52' ± 0.38	1.1812 ± 0.031	0.480 ± 0.009	0.567 ± 0.016
10	B4	24°.16' ± 0.63	1.2050 ± 0.035	0.473 ± 0.010	0.570 ± 0.015

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11	B5	20°.79' ± 0.59	1.1837 ± 0.019	0.528 ± 0.013	0.625 ± 0.021
12	B6	26°.22' ± 0.43	1.1860 ± 0.043	0.532 ± 0.016	0.631 ± 0.016

Table No. 3 Micromeritic properties of different batches of floating microspheres

Drug entrapment efficiency:

The drug entrapment efficiency of different batches of floating microspheres was found in the range of 63 % - 84 % w/w as shown in table 5. Drug entrapment efficiency was decreased with the increased drug concentration and increased with increasing polymer concentration in floating microspheres.The percentage yield of different batches was determined by weighing the floating microspheres after drying. The percentage yields of different formulation were in range of 68.6% - 77.5% Thepercentage yield of floating microspheres appeared unchanged by changing polymer ratio.

Table No. 4Entrapment efficiency ofdifferent batches of floating microspheres

Sr.No	Formulation code	Entrapment Efficiency(%)	Percentage Yield (%)
1	A1	74 ± 0.03	69.7 ± 0.02
2	A2	69.6 ± 0.02	68.6 ± 0.04
3	A3	84 ± 0.01	74.76 ± 0.03
4	A4	81.5 ± 0.04	75 ± 0.02
5	A5	73 ± 0.02	76.93 ± 0.02
6	A6	71.5 ± 0.03	77.5 ± 0.01
7	B1	72.8 ± 0.03	72.55 ± 0.04
8	B2	63 ± 0.02	69.9 ± 0.06
9	B3	79.5 ± 0.04	68.44 ± 0.08
10	B4	73 ± 0.06	69.16 ± 0.02
11	B5	78 ± 0.03	70.6 ± 0.04
12	B6	67± 0.06	70.23 ± 0.03

Scanning electronic microscopy:

The size and surface morphology of floating microspheres were examined by scanning electron microscopy as shown in figures. These Image No.1 &2 illustrating the microphotographs of formulation A3 and B3 at lower and higher magnification. The floating microspheres were spherical with no visible major surface irregularity. Few wrinkles and inward dents were appeared at the surface and some crystal shape particles were appeared. It may due to collapse of floating microspheres during the in- situ drying process. The surface morphology of both formulations was examined at higher magnification (600X)which illustrates the smooth surface of floating microspheres. Some small pores and cavities were present on the surface of floating microspheres, probably arising as a trace of solvent evaporation during the process.

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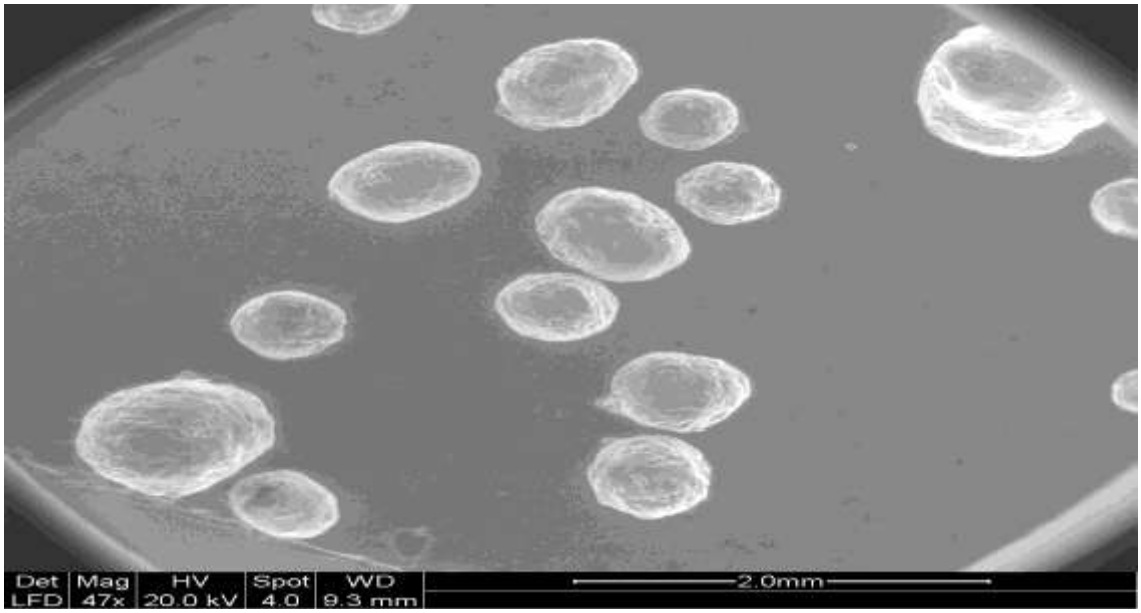

Figure No. 1 scanning electron microphotograph of formulation A3 at lower magnification

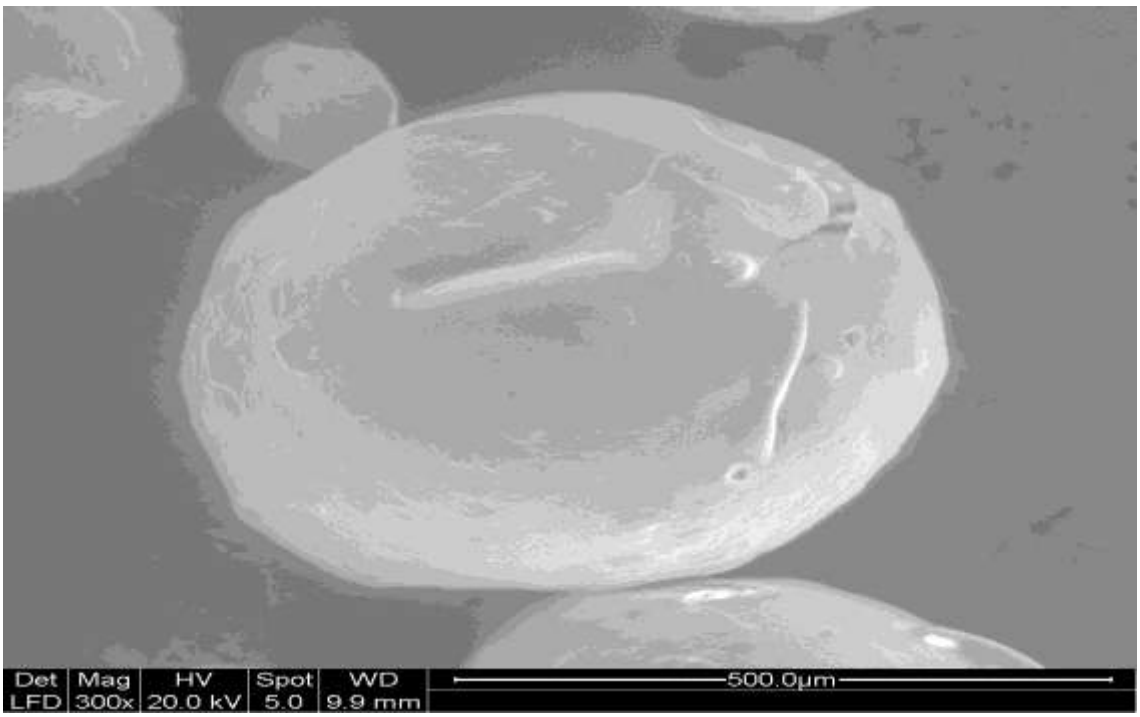

Figure No. 2 Scanning electron microphotograph of formulation A3 at higher magnification

Fourier transforms infrared spectroscopy (FT-IR) analysis

The FT-IR spectra of acyclovir, physical mixture of drug-polymer and floating microspheres (Batch A3) were recorded. The drug, acyclovir present in the formulation A3 was confirmed by FT-IR spectra. The characteristics peaks due to -OH, C=O, aryl alkyl ether and $–CH_2$ groups present in Acyclovir appeared in floating microspheres spectra (Batch A3), without any remarkable change in their position after successful encapsulation, indicating no chemical interaction between acyclovir and ethyl cellulose. It also confirmed the stability of drug during microencapsulation process.

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Table No. 5Fourier transforms infrared spectroscopy analysis

Transition	IR Range (cm^{-1})	Absorption wave number		
		Acyclovir	Physical Mixture	Formulation
O-H stretching vibration	3550 – 3200	3299.89	3200.03	3293.34
Aryl alkyl ether	1275 – 1200	1279.33	1279.58	1278.39
C=o stretching in guanine	1717	1718.43	1718.20	1718.59
CH_2 Scissoring	1485 – 1445	1486.12	1486.49	1486.28

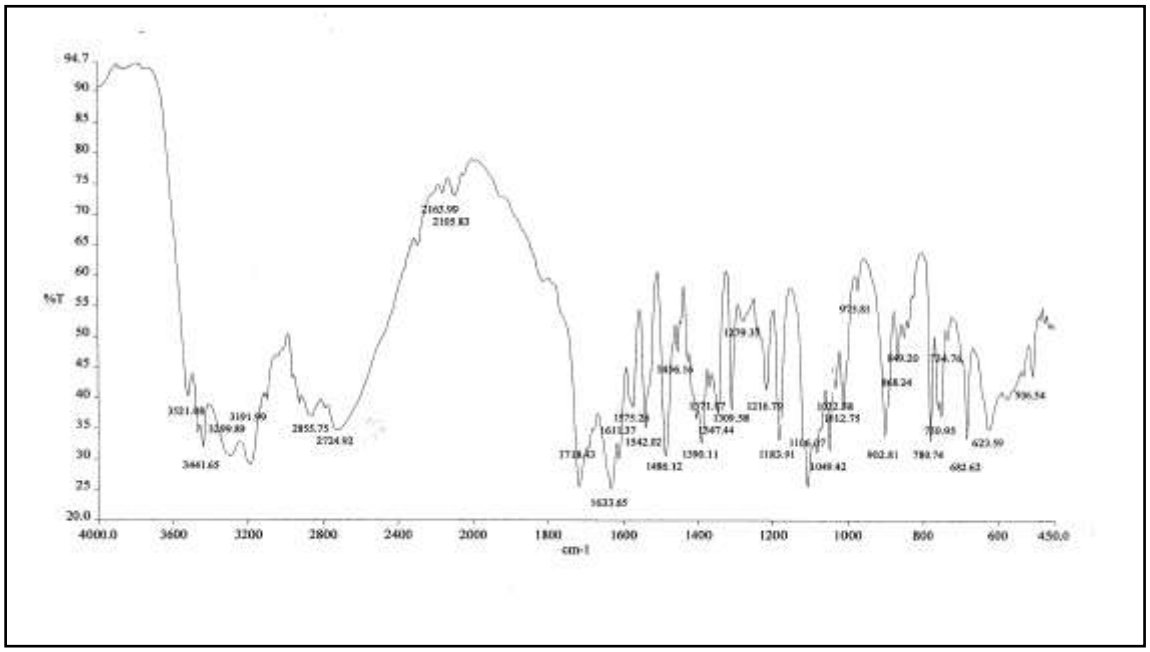

Figure No. 3FT-IR spectrum of acyclovir

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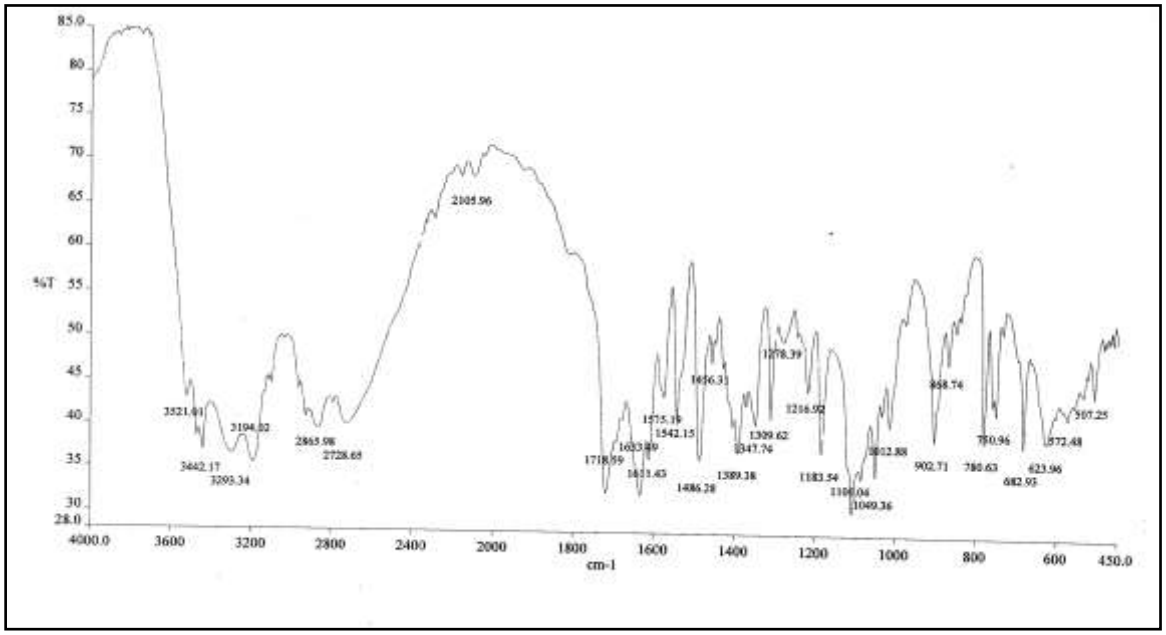

Figure No. 4FT-IR spectrum of floating microspheres (Batch A3)

Floating ability of floating microsphere:

Floating ability of different formulations was found to be differed according to polymer ratio. A1-A6 formulations showed best floating ability (62 – 36.87 %) in 8 hours. B1-B6 formulation showed less floating ability (47.12 - 32.16%) in 8 hours.

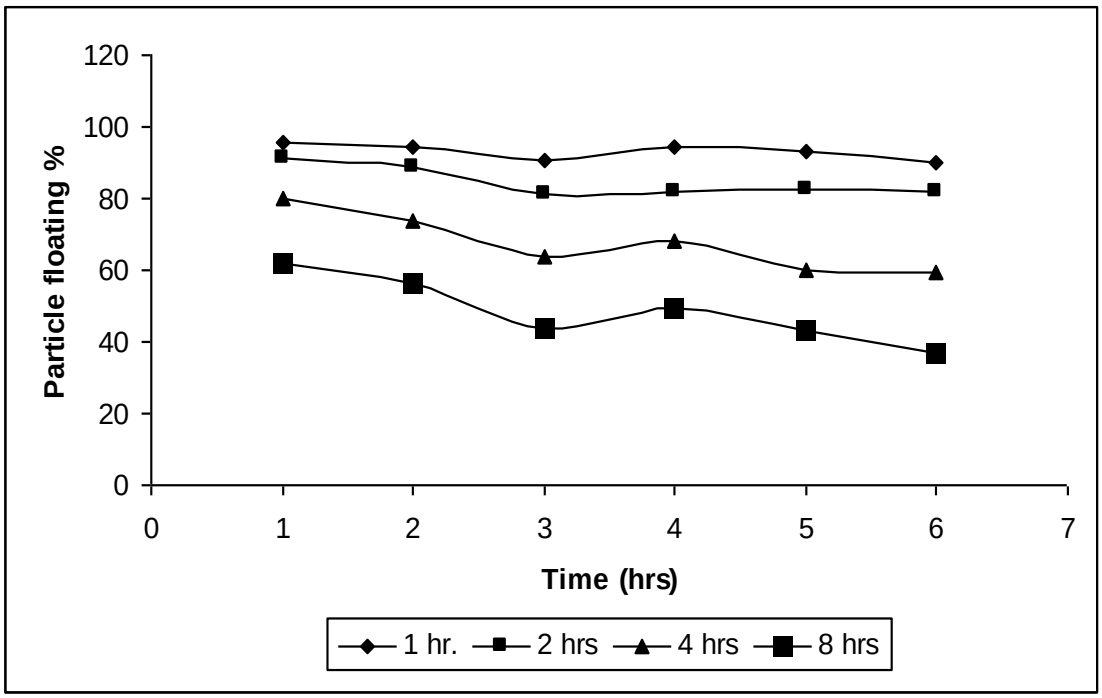

Figure No. 5Floating behaviour of formulation A1-A6

In-Vitro drug release study

Floating microspheres showed sustained release of the drug in acidic condition (pH 1.2) and the drug release was found to be approximately linear. Approximately 15% of the drug was released initially. Furthermore, drug release from

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the floating microspheres matrix was controlled by the polymer. Ethyl cellulose is not a water soluble polymer and it does not show pH dependency. As the polymer content was increased and the drug loading was decreased, the release of drug was decreased significantly.The effect of different plasticizer, (triethyl citrate) concentration (10 % and 20 %) on the release rate also studied. In the present floating microspheres formulation the plasticizer is used to render the wall material more elastic and flexible and never get fragile or ruptured under pressure. The release of drug was increased significantly with increasing plasticizer concentration from 10 to 20 %.In order to increase the release rate of drug, the ratio of polymer and plasticizer is decreased and increased respectively. Formulation A3 showed best appropriate balance between buoyancy and drug release rate. Release data has been shown in below table. Cumulative % release has been shown for average of three preparations.

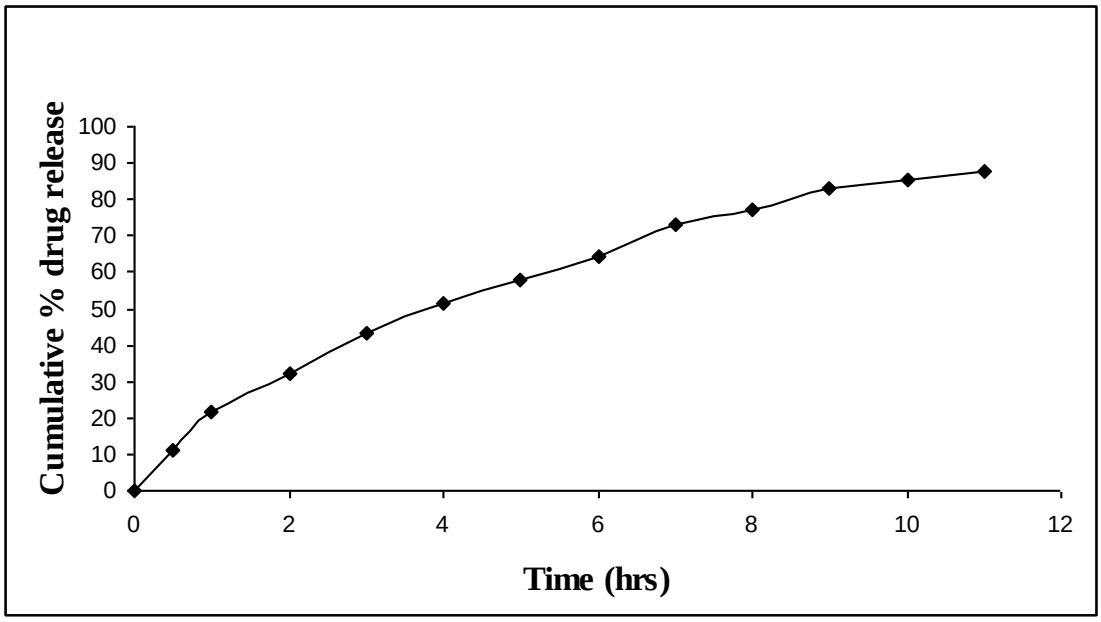

Figure No. 6 In vitro drug release profile of formulation A3 in 0.1 N HCI.

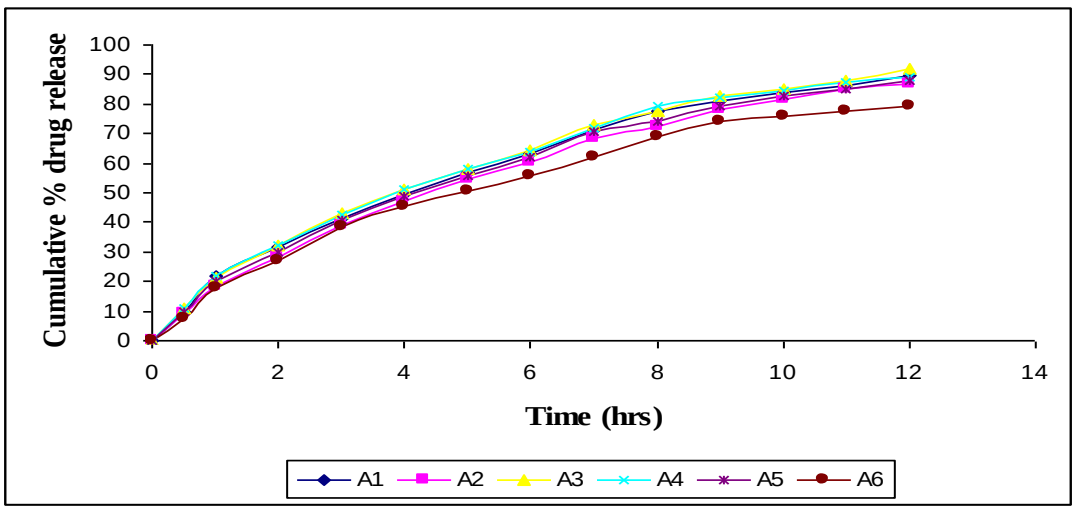

Figure No .7In vitro drug release profiles of formulations A1 –A6 in 0.1 N HCI

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Powder X-ray diffraction:

The X-ray powder diffractometry is widely used for identification of solid phases. The powder X-ray pattern of every crystalline form of a compound is unique. The powder X-ray pattern of Acyclovir and ethyl cellulose is shown in fig. 16 (a) and fig.16 (b). The X-ray pattern of acyclovir indicating its existence in crystalline form whereas, ethyl cellulose showed its existence in amorphous form. The X-ray pattern of microspheres (Batch A3) showed a combined pattern of those of the polymer and drug i.e. crystalline and amorphous. The diffraction peaks which are present in the drug X-ray pattern are also present in the formulation A3 X-ray pattern. These result indicated that acyclovir was present as drug crystal in the polymer matrix

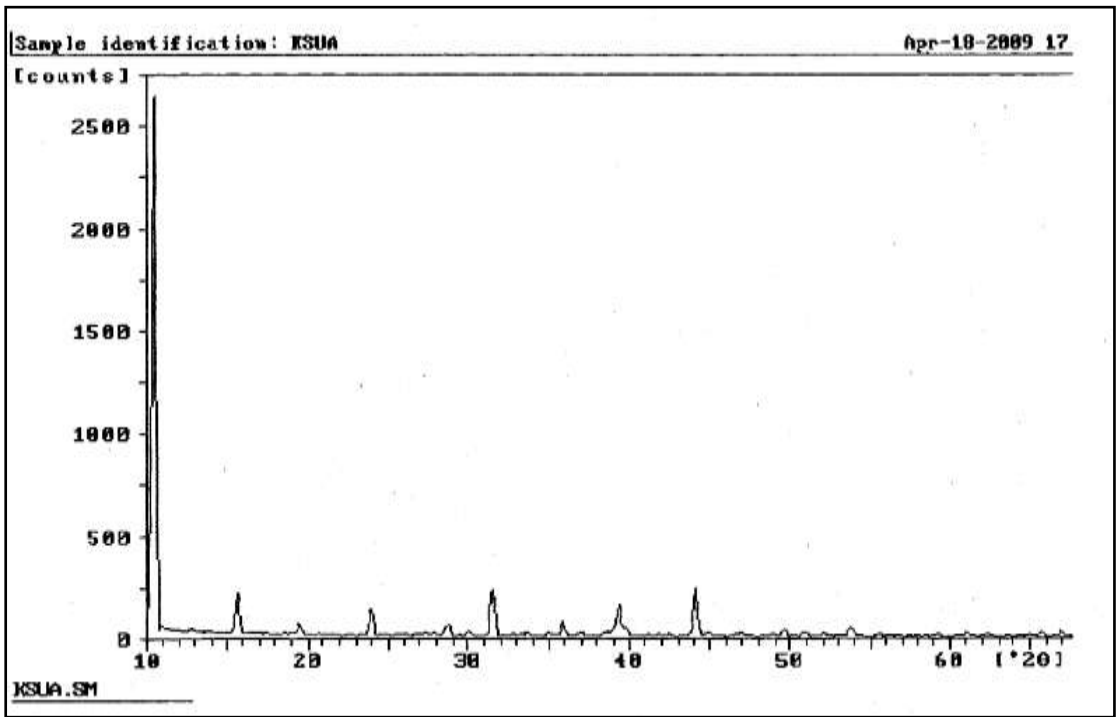

Fig. No:-8 X-ray pattern of ethyl cellulose

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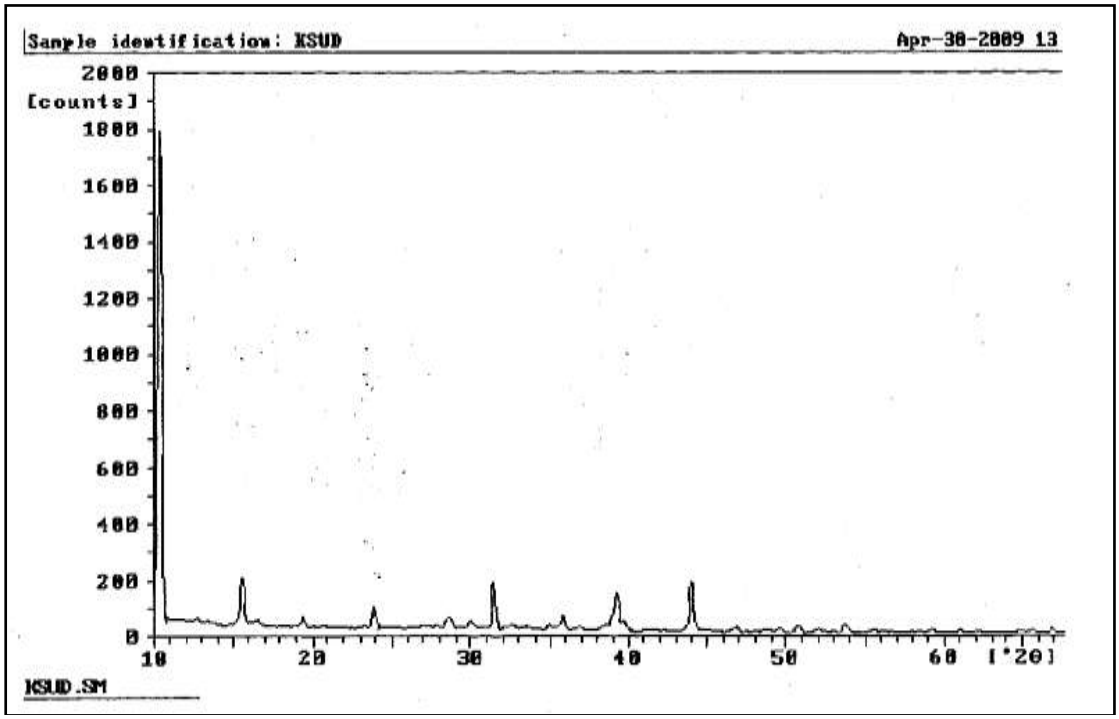

Fig. No:- 9 X-ray pattern of physical mixture

IV. CONCLUSION

The results obtained from this investigation are interesting and promising. The objective of the present investigation was to improve oral bioavailability of the poorly water soluble drug. For better absorption and enhanced bioavailability of some drug, prolongation of retention time of the dosage form in the stomach is essential. This problem can be solved by preparation of gastro-retentive drug delivery systems. An attempt was made to prepare floating microspheres of Acyclovir using ethyl cellulose. Ideal properties of floating microspheres include high buoyancy and sufficient release of drug in acidic condition. The prepared formulation (A3) showed best appropriate balance between buoyancy and drug release rate.

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