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Formulation and *In-Vitro* Evaluation of Floating Microspheres of Acyclovir

Hemangi S. Patil, Moreshwar P. Patil*, Bharat W. Tekade, Vinod M. Thakare, Vijay R. Patil.

T. V. E. S's Hon'ble Loksevak Madhukarrao Chaudhari College of Pharmacy,
Nehru Vidyanagar, Faizpur. (M.S), India.

The objective of this work was to prepare floating microspheres of acyclovir using different viscosities of ethyl cellulose to achieve an extended retention in upper GIT which may be result in enhanced absorption and thereby improves bioavailability. The floating microspheres were prepared by emulsion solvent diffusion technique and triethyl citrate was used as a plasticizer. The microspheres were evaluated for particle size analysis, drug entrapment, floating ability, *in-vitro* drug release and characterized by scanning electron microscopy and x-ray diffractometry. The mean particle size of all formulations was found in the range of 135.103 – 229.418 μm. The drug entrapment efficiency was in the range of 63 % - 84 % w/w. Floating ability of different formulations was found to be differed according to polymer ratio. The floating microspheres were spherical with no visible major surface irregularity. Few wrinkles and inward dents were appeared at the surface. The x-ray pattern of a formulation showed a combined pattern of those of the polymer and drug i.e. amorphous and crystalline respectively. The *in-vitro* release study indicated that when the polymer concentration was increased and the drug loading was decreased, the release of drug from microspheres was decreased.

Keywords: Acyclovir, Ethyl cellulose, Floating ability, Floating microspheres.

INTRODUCTION

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

Floating microspheres are gastro-retentive drug delivery systems based on non-effervescent approach. These microspheres are characteristically free flowing powders having a size less than 200 μm and remain buoyant over gastric contents and 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[3, 4].

Acyclovir, chemically it is 9–[(2 hydroxyethoxy) methyl] -9H- guanine 2-amino-1, 9-dihydro-9-[(2-hydroxyethoxy) methyl]-6H-purin-6-one. The drug is approved for the treatment of herpes simplex encephalitis, herpes genitalis, herpes labialis, herpes zoster, varicella (chickenpox), varicella-zoster virus and viral conjunctivitis. Acyclovir has a 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[5, 6]. The present work consists of preparation and evaluation of floating microspheres of acyclovir using ethyl cellulose of different viscosities in different proportions.

Corresponding Author

Moreshwar P. Patil

e-mail: morupatil@gmail.com

MATERIALS AND METHODS

Acyclovir, ethyl cellulose and triethyl citrate was received as a gift sample from Wockhardt Pvt. Ltd, Aurangabad. Dichloromethane and concentrated hydrochloric acid was purchased from Research Lab Ltd, Poona while polyvinyl alcohol Tween 20 was purchased from Qualigens Fine Chemicals, Mumbai and Loba Chemie Pvt. Ltd. Mumbai respectively. All other reagents used were of analytical grade.

For the study instruments like electronic balance (DC-9V, Sansui), pH meter (H196107, HANNA instrument, Italy), Double Beam UV Spectrophotometer (UV-1800, Shimadzu Co, Japan), USP dissolution test apparatus (TDT-08L, Electro Labs., Mumbai) and scanning electron microscopy (JSM-5610, JEOL Japan) were used.

Table 1. Composition of floating microspheres of Acyclovir

Sr. No	Formulation code	Acyclovir (gm)	Ethyl Cellulose (gm)		(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

Preparation of floating microspheres

Floating microspheres containing acyclovir were prepared by emulsion solvent diffusion technique using different viscosities of ethyl cellulose (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 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[7, 8]. The composition is given in Table 1.

Table 2. Particle size of floating microspheres

Formulation code	Mean particle size (μm) Mean±S.D
A1	152.531 ±2.85
A2	150.579 ± 3.53
A3	135.103 ± 1.43
A4	147.763 ± 3.12
A5	152.873 ± 2.17
A6	152.828 ± 1.86
B1	152.103 ± 2.16
B2	152.977 ± 3.26
B3	148.113 ±2.43
B4	229.418 ± 1.24
B5	147.965 ± 1.37
B6	150.676 ± 2.13

n=3

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. 1gm of floating microspheres was floated in 200 ml of aqueous solution of 0.02 % of Tween 20 and stirred at 37 ± 0.5 ^{0}C. 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) values of 10%, 50% and 90%. The mean particle size was then calculated[9].

Drug entrapment efficiency

The floating microspheres equivalent to 50 mg of acyclovir 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 suitable dilution the absorbance was measured at 254 nm using UV spectrophotometer and the percentage drug entrapped was calculated[9].

Floating ability of microspheres

Floating microspheres (50 mg) were placed in 0.1 N HCI (100 ml) containing 0.02% Tween 20 and stirred at 100 rpm. The layer of buoyant microspheres was pipette out and separated by filtration at 1, 2, 4 and 8 hours. The collected microspheres were dried in a desiccator over night. The percentage of microspheres was calculated[9].

In-vitro dissolution study

In-vitro dissolution 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 Acyclovir 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 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 spectrophotometricaly

Table 3. Evaluation of physical properties of floating microspheres

Formulation code	Angle of Repose (°) Mean±S.D	Bulk Denity (gm/cm³) Mean±S.D	Tapped Density (gm/cm³) Mean±S.D	% Compressibility index Mean±S.D
A1	17.91 ± 0.42	0.361 ± 0.005	0.428 ± 0.002	15.65 ± 0.696
A2	17.83 ±0.61	0.355 ± 0.007	0.422 ± 0.004	15.87 ± 0.543
A3	19.66 ± 0.20	0.385 ± 0.006	0.464 ± 0.008	17.02 ± 0.432
A4	19.81 ± 0.54	0.389 ± 0.007	0.474 ± 0.010	17.93 ± 1.465
A5	19.25 ± 0.48	0.422 ± 0.008	0.493 ± 0.009	14.40 ± 0.537
A6	20.26 ± 0.32	0.425 ± 0.007	0.490 ± 0.010	13.26 ± 0.693
B1	17.98 ± 0.61	0.447 ± 0.009	0.518 ± 0.013	13.70 ± 0.426
B2	22.64 ± 0.52	0.450 ± 0.006	0.522 ± 0.009	13.79 ± 1.231
B3	20.52 ± 0.38	0.480 ± 0.009	0.567 ± 0.016	15.34 ± 0.954
B4	24.16 ± 0.63	0.473 ± 0.010	0.570 ± 0.015	17.01 ± 0.742
B5	20.79 ± 0.59	0.528 ± 0.013	0.625 ± 0.021	15.52 ± 1.623
B6	26.22 ± 0.43	0.532 ± 0.016	0.631 ± 0.016	15.68 ± 0.882
n = 3				

at 254 nm to determine the amount of drug released in the dissolution medium[10].

Shape and surface characterization by SEM

From the formulated batches of floating microspheres, formulation (A3) and (B3) showed an appropriate balance between the buoyancy and the drug 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[11].

Characterization by x-ray diffractometry

Different samples were evaluated by X-ray powder diffraction. Diffraction patterns were obtained using x-ray diffractometer with a radius of 240 mm. The Cu Kα radiation (Kα 1.54060 Å) was Ni filtered. A system of diverging and receiving slits of 1° and 0.1 mm respectively was used. The pattern was collected with 40 kV of tube voltage and 30 mA of tube current and scanned over the 2θ range of $5–60^0$.

RESULTS AND DISCUSSION

Particle size analysis

The particle size of floating microspheres varied some what among the formulation due to variation in the composition of formulations. The mean particle size of floating microspheres formulation which shows high percentage of entrapment was in the range of 135-152 µm. Formulation B4 showed relatively higher percentage of large size and formulation A3 showed relatively small size floating microspheres.

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. The results are given in Table 2.

The results of physical properties of microspheres (Table 3) indicated that microspheres possess good flowability and compressibility[12, 13].

Table 4. Percentage yield and entrapment efficiency of floating microspheres

Formulation code	Percentage Yield	Entrapment Efficiency (%)
A1	**69.7 ± 0.02**	**74 ± 0.03**
A2	**68.6 ± 0.04**	**69.6 ± 0.02**
A3	**74.76 ± 0.03**	**84 ± 0.01**
A4	**75 ± 0.02**	**81.5 ± 0.04**
A5	**76.93 ± 0.02**	**73 ± 0.02**
A6	**77.5 ± 0.01**	**71.5 ± 0.03**
B1	**72.55 ± 0.04**	**72.8 ± 0.03**
B2	**69.9 ± 0.06**	**63 ± 0.02**
B3	**68.44 ± 0.08**	**79.5 ± 0.04**
B4	**69.16 ± 0.02**	**73 ± 0.06**
B5	**70.6 ± 0.04**	**78 ± 0.03**
B6	**70.23 ± 0.03**	**67 ± 0.06**
n=3		

Percentage yield and drug entrapment efficiency

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%

Drug entrapment efficiency was decreased with the increased drug concentration and increased with increasing polymer concentration in floating microspheres. This may be due to low solubility of acyclovir in water which facilitates the diffusion of a part of entrapped drug to surrounding medium during preparation of floating microspheres. The drug entrapment efficiency of different batches of floating microspheres was found in the range of 63 % - 84 % w/w. results are given in table 4.

Table 5. Floating ability of different batches of floating microspheres

Formulation code	1 hr.	2 hrs	4 hrs	8 hrs
A1	95.62	91	80	62
A2	94.33	89	74	56.33
A3	90.66	81.03	64	44
A4	94.25	82.16	68.27	49.66
A5	93.14	82.37	60.07	42.84
A6	90.07	81.66	59.67	36.87
B1	92.66	87.33	62.47	39.37
B2	95.22	82.59	65.09	47.12
B3	92.25	77.33	55.17	35.31
B4	93.12	78.22	58.27	32.16
B5	90.13	76.27	56.66	36.97
B6	92.41	81.66	56.71	38.84

n=3

Floating ability

The floating test was carried out to investigate the floating ability of the prepared microsphere. Floating ability of different formulations was found to be differed according to polymer ratio. A1-A6 formulations containing ethyl cellulose (50 cps) showed best floating ability than B1-B6 formulation containing ethyl cellulose (100 cps) in 8 hours. The results are given in Table 5.

***In-vitro* dissolution study** Ethyl cellulose is low permeable and water insoluble polymer. Floating microspheres showed sustained release of the drug in acidic environment and the drug release was found to be approximately linear. Approximately 15% of the drug was released initially. Furthermore, the drug release from the floating microspheres matrix was controlled by the polymer. As the polymer content was increased and the drug loading was decreased, the release of drug was decreased significantly. The effect of different concentration of triethyl citrate (10% and 20%) on the release rate also

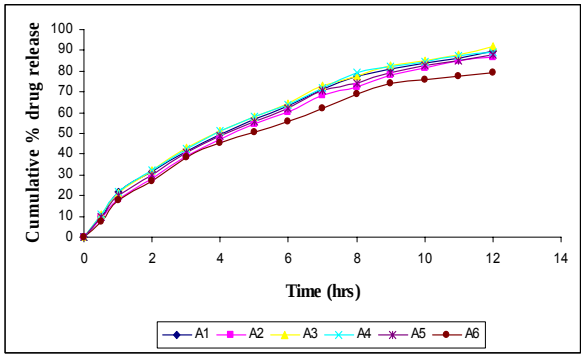

Fig. 1 *In vitro* drug release profile of formulations A1 –A6 in 0.1 N HCI

studied. It was 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.

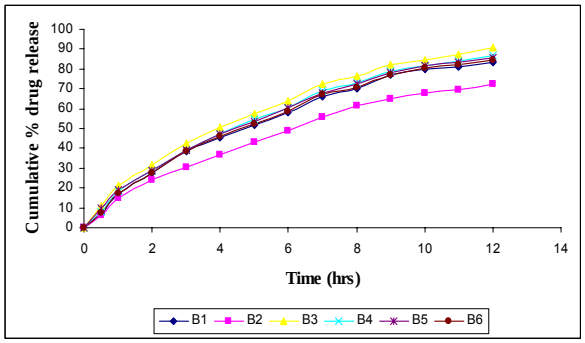

Fig. 2 *In vitro* drug release profile of formulations B1 –B6 in 0.1 N HCI

The size and surface morphology of floating microspheres were examined by scanning electron microscopy as shown in figures (Figure 3, 4, 5 and 6) illustrating the microphotographs of formulation A3 and B3. 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 which illustrates the smooth surface of floating microspheres. Some small

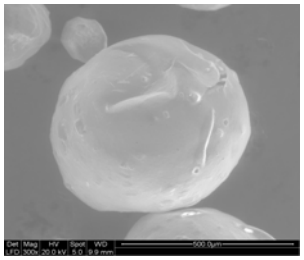

Fig. 3 Scanning electron microphotograph of formulation A3

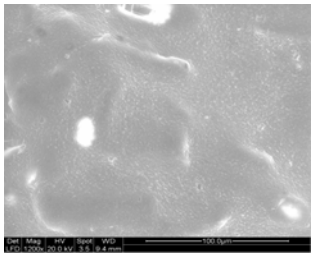

Fig. 4 Scanning electron microphotograph of surface morphology of formulation A3

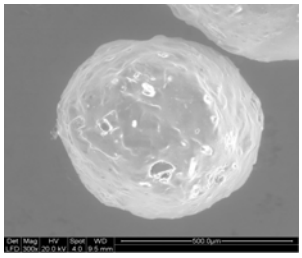

Fig. 3 Scanning electron microphotograph of formulation B3

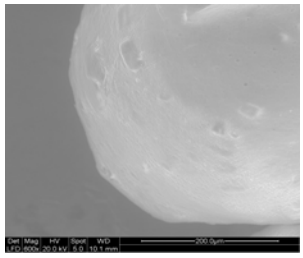

Fig. 4 Scanning electron microphotograph of surface morphology of formulation B3

pores and cavities were present on the surface of floating microspheres, probably arising as a trace of solvent evaporation during the process.

Characterization by x-ray diffractometry

The powder X-ray pattern of every crystalline form of a compound is unique. The powder X-ray pattern of Acyclovir, ethyl cellulose, their physical mixture and formulation is shown in figure 7. The X-ray pattern of acyclovir indicated its existence in crystalline form whereas, ethyl cellulose showed its existence in amorphous form. The diffraction pattern of their physical mixture is a sum of their combination. The X-ray pattern of formulation showed a combined pattern of those of the polymer and drug i.e. crystalline and amorphous. The drug is dispersed in the polymer matrix

CONCLUSION

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. From the results it can be concluded that the drug release from the floating microspheres matrix was controlled by the polymer. When the polymer proportion in the formulation was increased with decreased drug

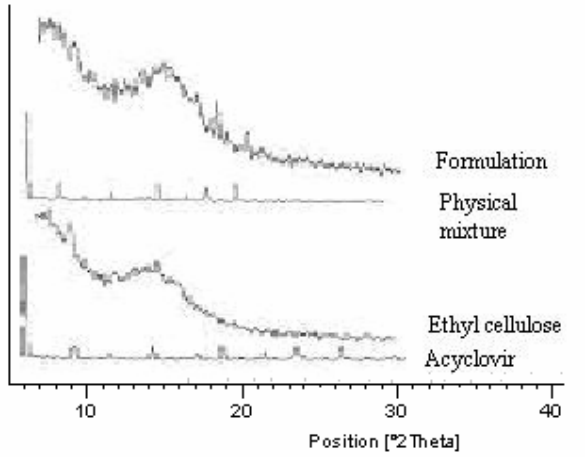

Fig. 7. X-ray diffraction pattern of Acyclovir, ethyl cellulose (EC), physical mixture and formulation

loading, drug release was decreased significantly. Addition of plasticizer made the wall of material more elastic and flexible and never gets fragile or ruptured under pressure. It was also observed that the release of drug was increased significantly with increasing plasticizer concentration. The prepared formulation showed appropriate balance between buoyancy and drug release rate.

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