ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 7 | Issue: 8 | August 2021|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2021: 8.047 || ISI Value: 1.188

PREPARATION OF FLOATING MICROSPHERES OF RITONAVIR BY EMULSION SOLVENT DIFFUSION TECHNIQUE

Dr.G.Jagdish[1], Abhay Mishra[2], Mohammad Akhtar Rasool[3]

[1]*Professor and Principal, DBM College of Pharmacy,Janjgir Chattisgarh*

[2]*Assistant Professor DBM College of Pharmacy,Janjgir Chattisgarh*

[3]*Associate Professor, J K College of Pharmacy,Bilaspur*

ABSTRACT

In recent years scientific and technological advancements have been made in the research and development of rate-controlled drug delivery system by overcoming physiological adversities such as short gastric residence time (GRT) and unpredictable gastric emptying time (GET). Several approaches are currently utilized in the prolongation of GRT, including floating drug delivery system (FDDS), also known as hydrodynamically balanced system (HBS), swelling and expanding system, polymeric bioadhesive system, modified shape system, high density system, and other delayed gastric emptying devices 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 Ritonavir 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 technique is 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), Ritonavir, Ethyl cellulose, Gastric emptying time (GET), Microspheres.***

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 complete release 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

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 7 | Issue: 8 | August 2021|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2021: 8.047 || ISI Value: 1.188

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]

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.

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 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.[4]

MATERIALS

The following materials were used for the research work. All chemicals used were of best quality available. Ritonavir, Ethyl cellulose, Triethyl Citrate was obtained as kind gift sample from Wockhardt 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 Floating Microspheres of Ritonavir by Emulsion Solvent Diffusion Technique

Floating microspheres containing Ritonavir 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.

Table No. 1 Formulation of the floating microspheres of Ritonavir

Sr. No	Formulation code	Drug (Ritonavir) (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

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 7 | Issue: 8 | August 2021|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2021: 8.047 || ISI Value: 1.188

EVALUATION OF MICROSPHERES

1. 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 of floating 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) values of 10%, 50% and 90%. The mean particle size was then calculated.[5]

2. Percentage Yield

The percentage yield of different formulations was determined by weighing the floating microspheres after drying. The percentage yield was calculated as follows.[6]

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

3. Drug Entrapment:

The various batches of the floating microspheres were subjected to estimation of drug content. The floating microspheres equivalent to 50 mg of Ritonavir 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.[5]

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

4. 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.

5. 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 Ritonavir, Ethyl Cellulose and floating microspheres were recorded.[5]

6. Powder X-ray diffraction

The powder X-ray diffraction pattern of Ritonavir 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.

7. 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]

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

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 7 | Issue: 8 | August 2021|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2021: 8.047 || ISI Value: 1.188

8. *In-vitro* release studies

In-vitro release of Ritonavir 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 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.

RESULTS

1. 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

2. Angle of repose

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.

Table No. 3 Angle of repose of different batches of floating microspheres

Sr.No	Formulation code	Angle of Repose (°)
1	A1	17°.91' ± 0.42
2	A2	17°.83' ±0.61
3	A3	19°.66' ± 0.20
4	A4	19°.81' ± 0.54
5	A5	19°.25' ± 0.48
6	A6	20°.26' ± 0.32
7	B1	17°.98' ± 0.61
8	B2	22°.64' ± 0.52
9	B3	20°.52' ± 0.38
10	B4	24°.16' ± 0.63
11	B5	20°.79' ± 0.59
12	B6	26°.22' ± 0.43

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 7 | Issue: 8 | August 2021|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2021: 8.047 || ISI Value: 1.188

3. Hausner's Ratio

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.

Table No. 4 Hausner's Ratio values of different batches of floating microspheres

Sr.No	Formulation code	Hausner's Ratio
1	A1	1.1855 ± 0.023
2	A2	1.1887 ± 0.018
3	A3	1.2051 ± 0.020
4	A4	1.2185 ± 0.016
5	A5	1.1682 ± 0.025
6	A6	1.1529 ± 0.032
7	B1	1.1588 ± 0.028
8	B2	1.16 ± 0.042
9	B3	1.1812 ± 0.031
10	B4	1.2050 ± 0.035
11	B5	1.1837 ± 0.019
12	B6	1.1860 ± 0.043

4. 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.

Table No. 5 Entrapment efficiency of different batches of floating microspheres

Sr.No	Formulation code	Entrapment Efficiency (%)
1	A1	74 ± 0.03
2	A2	69.6 ± 0.02
3	A3	84 ± 0.01
4	A4	81.5 ± 0.04
5	A5	73 ± 0.02
6	A6	71.5 ± 0.03
7	B1	72.8 ± 0.03
8	B2	63 ± 0.02
9	B3	79.5 ± 0.04
10	B4	73 ± 0.06
11	B5	78 ± 0.03
12	B6	67 ± 0.06

5. 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.

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 7 | Issue: 8 | August 2021|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2021: 8.047 || ISI Value: 1.188

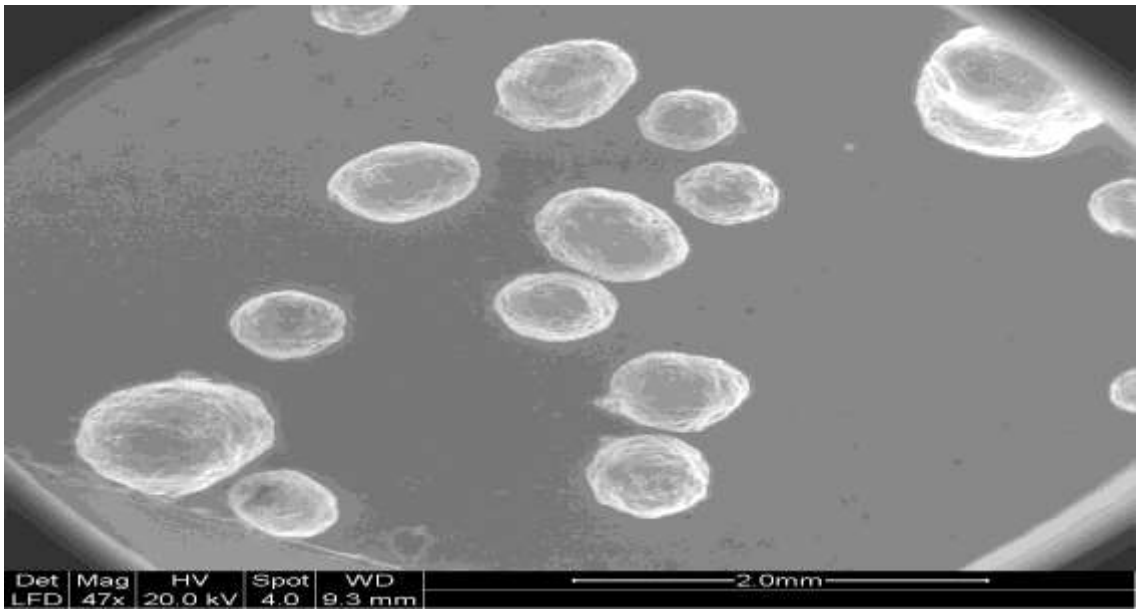


Image No. 1 scanning electron microphotograph of formulation A3 at lower magnification

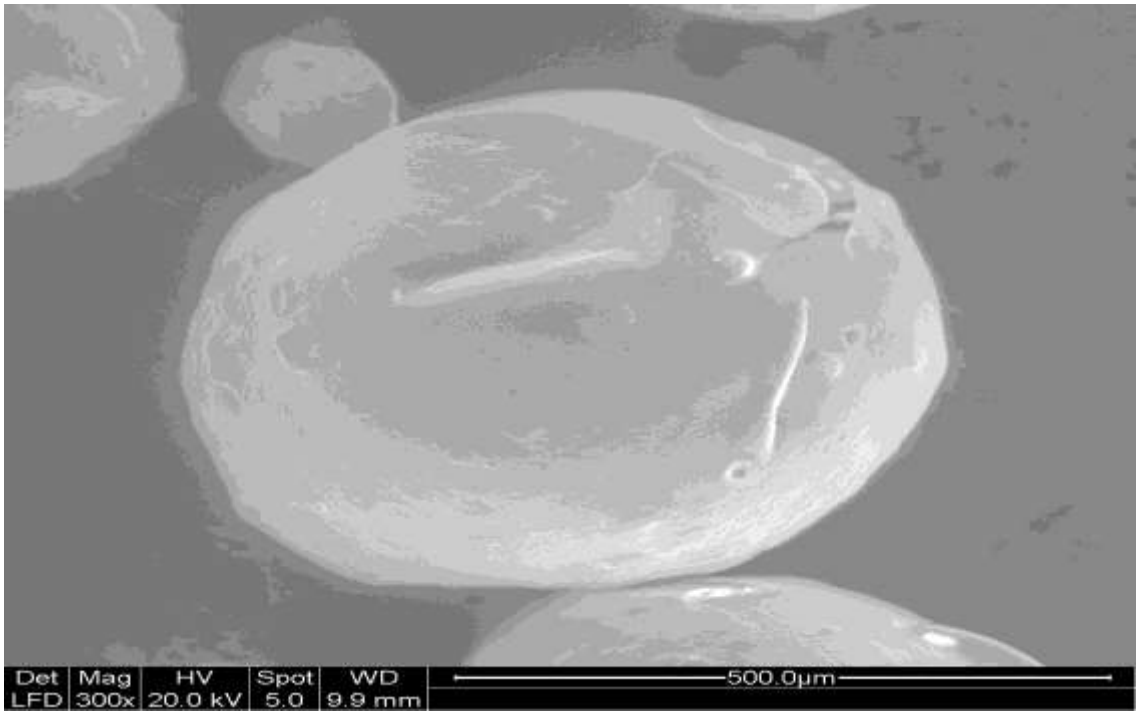
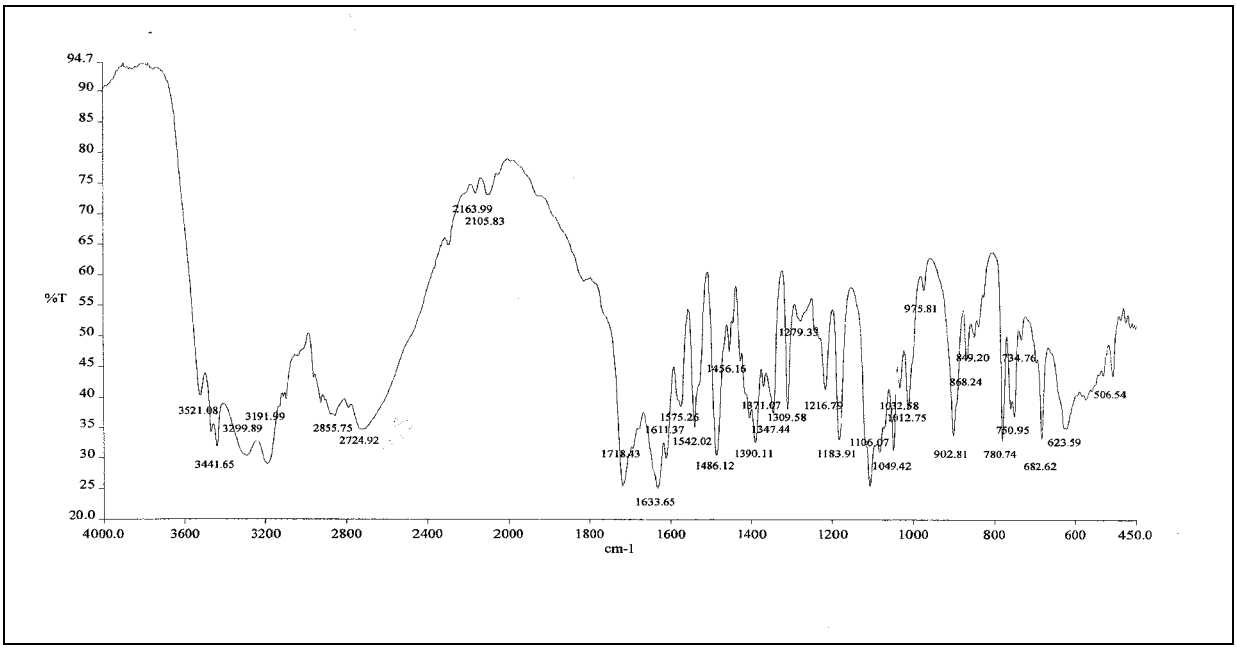


Image No. 2 Scanning electron microphotograph of formulation A3 at higher magnification

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 7 | Issue: 8 | August 2021|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2021: 8.047 || ISI Value: 1.188

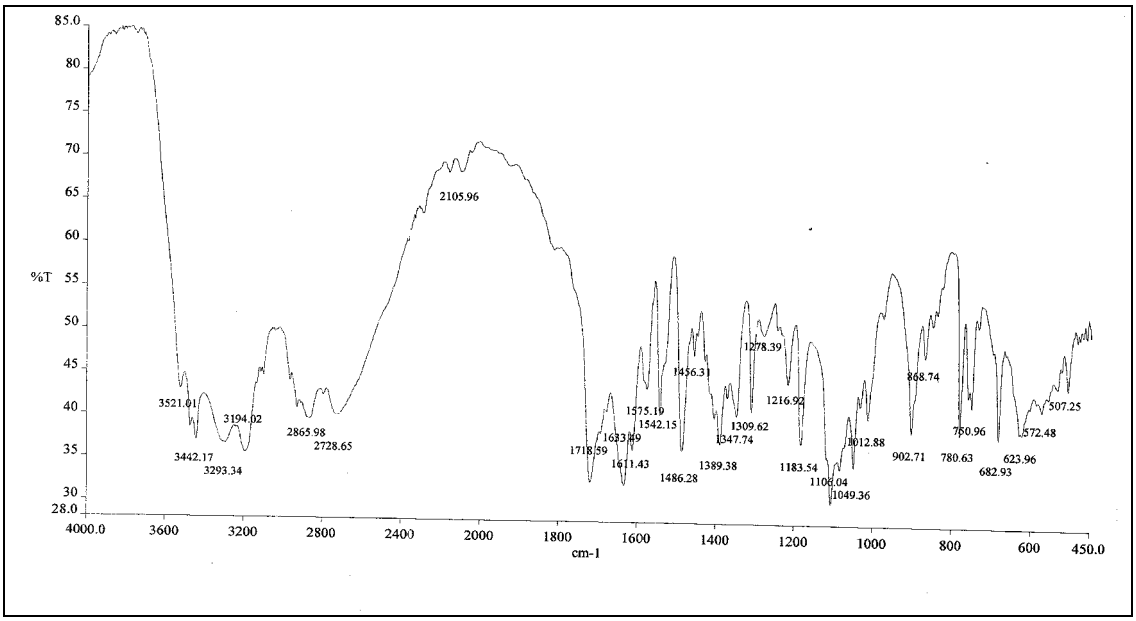
Table No. 6 Fourier transforms infrared spectroscopy (FT-IR) analysis Interpretation of FT-IR

Transition	IR Range (cm^{-1})	Absorption wave number		
		Ritonavir	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



Graph No. 1 FT-IR spectrum of Ritonavir

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 7 | Issue: 8 | August 2021|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2021: 8.047 || ISI Value: 1.188



Graph No. 2 FT-IR spectrum of floating microspheres (Batch A3)

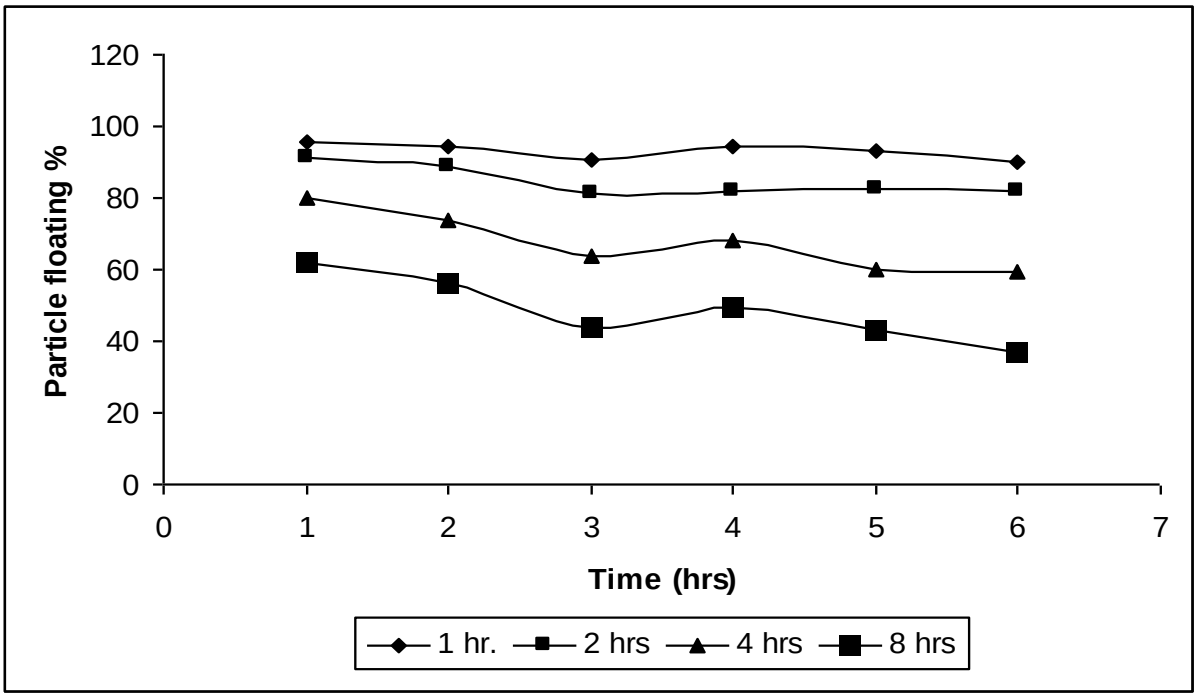
6. Floating ability of floating microsphere:

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 as showed in Table-07

Table No. 7 The percentage floating ability of different batches of floating microspheres

Sr.No.	Formulation code	1 hr.	2 hrs	4 hrs	8 hrs
1	A1	95.62	91	80	62
2	A2	94.33	89	74	56.33
3	A3	90.66	81.03	64	44
4	A4	94.25	82.16	68.27	49.66
5	A5	93.14	82.37	60.07	42.84
6	A6	90.07	81.66	59.67	36.87
7	B1	92.66	87.33	62.47	39.37
8	B2	95.22	82.59	65.09	47.12
9	B3	92.25	77.33	55.17	35.31
10	B4	93.12	78.22	58.27	32.16
11	B5	90.13	76.27	56.66	36.97
12	B6	92.41	81.66	56.71	38.84

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 7 | Issue: 8 | August 2021|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2021: 8.047 || ISI Value: 1.188



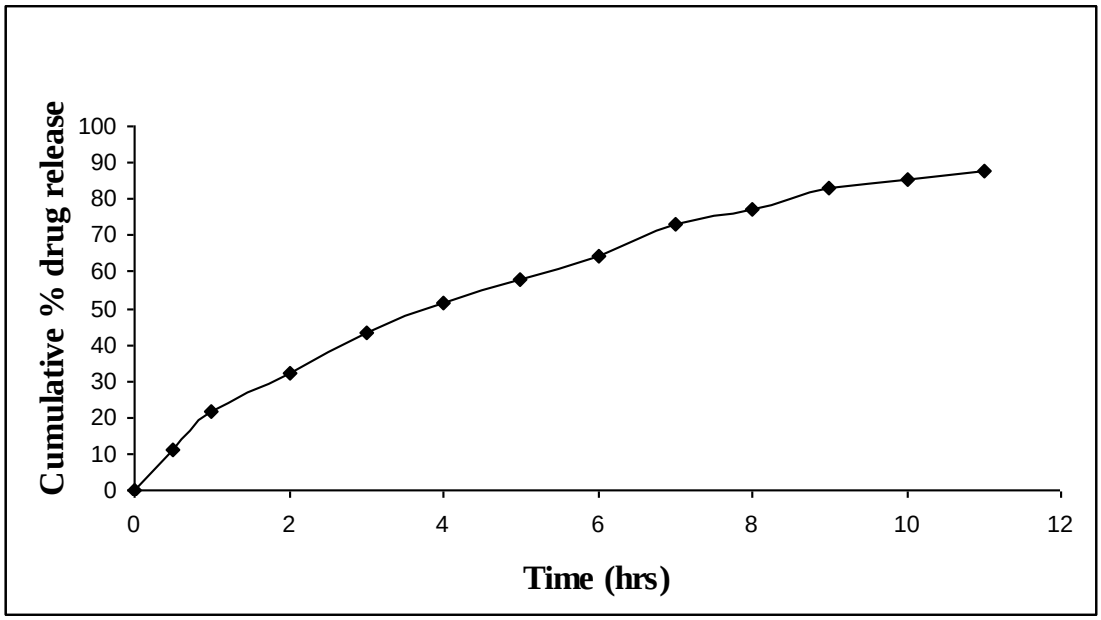
Graph No. 3 Floating behaviour of formulation A1-A6

***7. In-Vitro* drug release study**

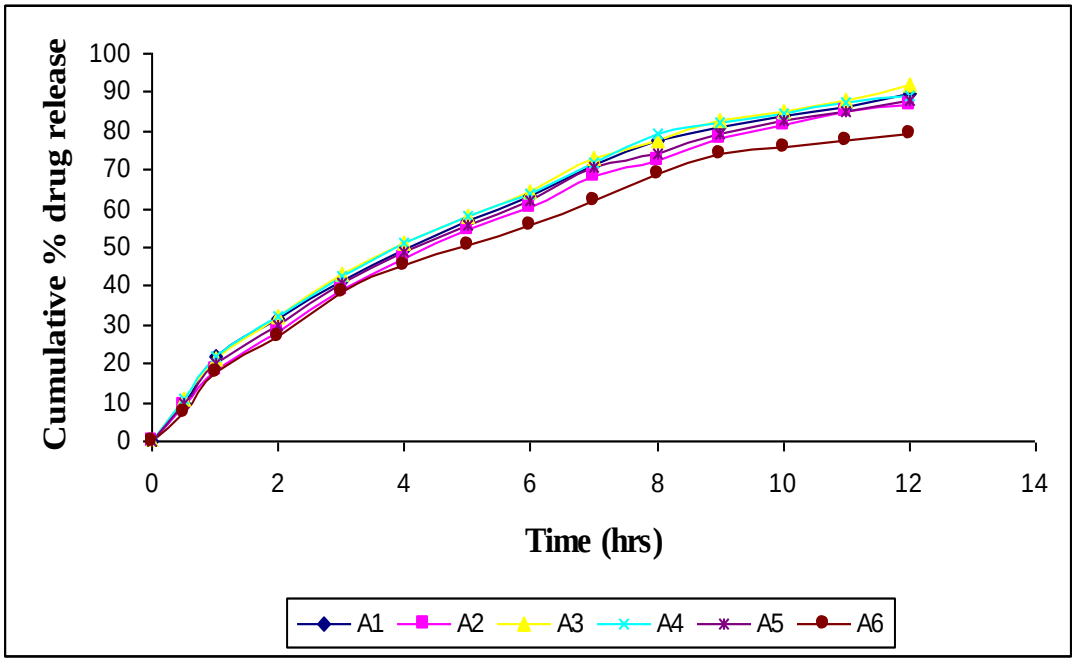
Table No. 8 *In vitro* dissolution profile of formulation A3 in 0.1 N HCI.

Sr. No.	Time (hrs)	Absorbance at 254 nm	Cumulative % release ±S.D
1	0	0	0
2	0.5	0.145	10.878 ± 0.912
3	1	0.251	21.477 ± 1.263
4	2	0.357	32.193 ± 1.621
5	3	0.464	43.124 ± 0.873
6	4	0.543	51.405 ± 0.945
7	5	0.604	57.993 ± 1.102
8	6	0.664	64.549 ± 1.171
9	7	0.745	73.247 ± 0.759
10	8	0.779	77.388 ± 0.886
11	9	0.826	82.851 ± 1.235
12	10	0.841	85.203 ± 0.935
13	11	0.859	87.868 ± 0.864
14	12	0.891	91.936 ± 1.610

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 7 | Issue: 8 | August 2021|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2021: 8.047 || ISI Value: 1.188



Graph No. 4 *In vitro* drug release profile of formulation A3 in 0.1 N HCI.



Graph No. 5 *In vitro* drug release profiles of formulations A1 –A6 in 0.1 N HCI

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 Ritonavir using ethyl cellulose. Ideal properties of floating microspheres include

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 7 | Issue: 8 | August 2021|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2021: 8.047 || ISI Value: 1.188

high buoyancy and sufficient release of drug in acidic condition. The prepared formulation (A3) showed best appropriate balance between buoyancy and drug release rate.

REFERENCES

1. *Robinson, J. R., Lee, V. H. L., 1987. Controlled drug delivery fundamentals and applications. 2nd ed. Marcel Dekker, Inc, New York, pp. 418-421*
2. *Hajare, A. A., Shetty, Y. T., 2008. Formulation, characterization and in – vitro evaluation of floating microspheres of diltiazem hydrochloride by ionotropic gelation technique. Res. J. Pharm. Tech. (1), 52 – 56.*
3. *Singh, B. N., Kim, K. H., 2000. Floating drug delivery system: An approach to oral controlled drug delivery via gastric retention. J. Control. Release 63 (3), 235-259.*
4. *Tanwar, Y. S., 2006. Floating microspheres: Development, Characterization and application. 4(3), www.pharmainfo.net , 15-2-2009.*
5. *Junyaprasert, V.B., Pornsuwannapha, S., 2008. Floating properties and release characteristics of hollow microspheres of Ritonavir. Drug Delivery 15, 331-341.*
6. *Addanki Gopikrishna, B. Ramu, G. Srikanth, Dr. Bigala Rajkamal (2016). Formulation of isoniazide sustained release formulation by using carbopol 934 P P. Int J App Pharm Sci Res. 1(2):60- 69. Doi: 10.21477/ijapsr.v1i2.10177.*
7. *B. Ramu, Kaushal K. Chandrul, P. Shanmuga Pandiyan. Using 24 Factorial Designs optimization of Repaglinide Gastroretentive Drug Delivery System. Research J. Pharm. and Tech. 2021; 14(2):725-729. doi: 10.5958/0974-360X.2021.00126.8*
8. *B Ramu, N. Ramakrishna, Meruva Sathish, D. Anoosha (2015). Formulation of tellmisartan Hcl Fast Disintegrating Tablets by Sublimation Technique. International Journal of Pharm Tech Research. 8(3), 330-339.*
9. *Trivedi, P., Verma, A. M. L., Garud, N., 2008. Preparation and characterization of aceclofenac microspheres. Asian J. Pharm. 110-114.*