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Original Research Article

Study of Stavudine Multiparticulate Floating Drug Delivery System Prepared by Emulsion Gelation Technique

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Abstract: Gastroretentive systems can remain in the gastric region for several hours and hence significantly prolong the gastric residence time of drugs. Prolonged gastric retention improves bioavailability, reduces drug waste, and improves solubility for drugs that are less soluble in a high pH environment. The present work describes the formulation and evaluation of gastroretentive system of an antiretroviral agent, Stavudine, based on the concept of altered density. Emulsion gelation technique was used to prepare the floating microcarriers using sodium alginate as the polymer. Microcarriers containing oil was prepared by gently mixing and homogenizing oil and water phase containing sodium alginate which was then extruded into calcium chloride solution. The prepared microcarriers were evaluated for drug entrapment efficiency, particle size and shape, micrometric properties, buoyancy and in-vitro drug release studies. The results of FTIR spectroscopy showed stable character of Stavudine. The mean particle size of microcarriers was in the range of 0.59-1.25mm. Microcarriers were spherical and free flowing. The drug entrapment efficiency was found to be 44.6-69.1%. The microcarriers remained buoyant for more than about 12h. The drug release study showed that Stavudine from the microcarriers was prolonged more than 10hrs. The results demonstrate that the amount of the oil entrapped in each microcarrier is play role in particle size entrapment efficiency and in vitro drug release.

Keywords: Floating drug delivery system, Emulsion gelation, Stavudine, Calcium alginate, Mineral oil

INTRODUCTION

Absorption window in the proximal gut can limit the bioavailability of orally administered compounds and can be a major obstacle to the development of controlled release formulations for important drugs. Two main approaches are presently being explored: (i) bioadhesive microspheres that have a slow intestinal transit; and (ii) the gastroretentive dosage system, which is based on multiparticulates or large single unit systems. A good understanding of gastrointestinal transit in humans and the effect of factors such as food can be helpful in the design of rational systems that will have clinical benefit. Gastric retentive delivery systems potentially allow increased penetration of the mucus layer and therefore increase drug concentration at the site of action. These systems can remain in the gastric region for several hours and hence significantly prolong the gastric residence time of drugs. Prolonged gastric retention improves bioavailability, reduces drug waste, and improves solubility for drugs that are less soluble in a high pH environment. It has applications also for local drug delivery to the stomach and proximal small intestines. Gastroretention helps to provide better availability of new products with new therapeutic possibilities and substantial benefits for patients [1].

With the aim of the development of oral-controlled release dosage forms, it has attracted much attention on the polymers that can control the release of drugs such as polymeric hydrogels, which are being increasingly investigated for controlled release applications because of their good compatibility. In addition, the ability of hydrogels to release an entrapped drug in aqueous medium and to regulate the release of such drug by control of swelling and by cross-linking makes them particularly suitable for controlled release applications. Hydrogels can be applied for the release of both hydrophobic and hydrophilic drugs and charged solutes. Gastroretentive microparticles have been investigated, but few studies have demonstrated success in clinical investigations. Pivotal studies in Nottingham University, UK, have revealed that oral dosage forms containing finely divided ion-exchange resins can provide prolonged gastric residence and uniform distribution within the stomach. For such an effect, the particles will need to be small from a mechanical consideration and of low density so that they might be able to float . Several approaches like floating multiparticulates system using ion exchange resin loaded with bicarbonate5 , floating beads of riboflavin using sodium alginate solution containing CaCO3 or NaHCO3 as gas generating agentF6 , piroxicam in hollow polycarbonate (PC) micro spheres, hollow microspheres (microballoons) loaded with Ibuprofen in an outer enteric acrylic polymer , air compartment multiple-unit system for prolonged gastric residence , microspheres by core solubilization technique, wax and fat embedded floating micro spheres of Ibuprofen, floating microspheres by different solvent evaporation technique, floating bioadhesive microspheres containing acetohydroxamic acid by quasi-emulsion solvent diffusion method, Dry Coated Drug Delivery System With Floating-Pulsatile Release were developed and studied for there gastroretentive properties [2].

Stavudine (D4T, thymidine) is a thymidine analog and chemically known as 1-[(2R,5S)-5-(hydroxymethyl)-2,5-dihydrofuran-2-yl]-5-methyl-1,2,3-tetrahydropyrimidine-2,4dione and it is FDA-approved drug for clinical use for the treatment of HIV infection [3]. Stavudine is administered either alone or in combination with other antiviral agent. Stavudine upon phosphorylated using cellular kinases to reactive metabolite stavudine triphosphate. Stavudine triphosphate inhibits the activity of HIV-1 reverse transcriptase by competing with the natural substrate thymidine triphosphate and by causing DNA chain termination following its incorporation into viral DNA. Stavudine triphosphate inhibits cellular DNA polymerases β and γ and markedly reduces the synthesis of mitochondrial DNA. Stavudine is typically administered orally as a capsule and an oral solution. The drug has a very short half-life (1.30 h) thus necessitating frequent administration to maintain constant therapeutic drug levels. However patients receiving Stavudine develop neuropathy and lacticacidosis. The side effects of Stavudine are dose-dependentand a reduction of the total administered dose reduces the severity of the toxicity.

In the present investigation we developed an extended and controlled release composition and formulation of Stavudine using expandable, gelling, swellable hydrocolloid polymer along with the mineral oi [3]. The polymer used was sodium alginate, which is an inexpensive, nontoxic product extracted from kelp. Sodium alginate has been used as thickening and gelling agent. Additionally it also reduces interfacial tension between an oil and water phase and is efficient for preparation of emulsion.

MATERIALS AND METHODS

Materials

Stavudine was received as a gift sample from Doctors Life Sciences, Nellore (India), Liquid paraffin was purchased from Himalaya Chemicals Ltd., Hyderabad (India), Sodium alginate was purchased from Himalaya Chemicals Ltd., Hyderabad (India), and all other chemicals used were of analytical grade.

Methods

Preparation of oil-entrapped microcarriers by Emulsion gelation method

Table 1: Formulation of Stavudine floating microcarriers

Formulation	Stavudine	Sodium alginate	Liquid paraffin	Distilled water
F1	300mg	2.5g	2.5g	50ml
F2	300mg	2.5g	5g	50ml
F3	300mg	2.5g	7.5g	50ml
F4	300mg	2.5g	10g	50ml
F5	300mg	2.5g	12.5g	50ml
F6	300mg	2.5g	15g	50ml

Formulations F1-F6 was prepared by emulsion gelation method. Sodium Alginate (5%) was dissolved in distilled demineralized water with agitation. Stavudinne and different concentrations of mineral oil were added to the solution. This solution containing Stavudine (300 mg) and oil was dropped through 21 G needle in to 1% calcium chloride (10 ml) and left at room temperature for 2 h. The resultant microcarriers were washed twice with distilled water and kept for drying at room temperature up to 12 hours [4-6].

EVALUATION OF FLOATING MICROCARRIERS

a) Floating behaviour

300 mg of the dried micreocarriers were spread over the surface of USP XXIV dissolution apparatus type II. Simulated gastric fluid without enzyme of pH 1.2 was used as medium (900 ml) and was medium maintained at 37^{o}C ± 0.5^{o} C for 12 hrs. The paddle speed was controlled at 100 rpm. The floating and the settled portion of microparticles were recovered separately. After drying, each fraction of the microparticulates was weighed and their buoyancy was calculated by the following equation [7]:

Buoyancy (%) = Qf / Qf + Qs

b) Particle size

The mean diameter of 100 microparticles was determined by optical microscopy (Metzer, India). The optical microscope was fitted with a stage micrometer by which the size of microcarriers could be determined [8].

c) Drug entrapment efficiency

Stavudine drug content in the floating microcarriers was calculated by UV spectrophotometric method. The method was validate for linearity, accuracy and precision. A sample of dried microcarriers equivalent to 100 mg was taken in to mortar and pestle and add little amount of phosphate buffer of pH 7.4 and triturated for 7 to 10 minutes. Then transfer content in to 100 ml volumetric flask and make up volume to 100 ml with phosphate buffer of pH 7.4. The solution was filtered through whatman filter paper. From the resulting solution take 1 ml in to 100 ml volumetric flask and then make up volume to 100 ml with phosphate buffer of pH 7.4. Drug content was determined by UV spectrophotometer at 266 nm. The entrapment was calculated by using following formula [9-11].

$$\textbf{Entrapment efficiency} = \frac{\textbf{Actual drug content}}{\textbf{Theoretical drug content}} \times 100$$

d)Scanning electronic microscopy (SEM)

The shape and surface characteristics were determined by scanning microscopy (model- JSM, joel, japan) using gold sputter technique. The particles were vacuum dried, coated to 200 nm thickness with gold palladium using prior to microscopy. A working distance of 20nm, a tilt of zero- degree and accelerating voltage of 15kv were the operating parameters. Photographs were taken within a range of 50-500 magnification [12].

e) In-vitro drug release study

In vitro release rate studies were carried out using XXIV apparatus type I. Simulated gastric fluid without enzymes of pH 1.2 was used as dissolution medium (900 ml) and was maintained at $37^{o}C \pm 0.5^{o}C$. Approximately 0.1 g microcarriers were used for each experiment. The paddle speed was controlled at 50 rpm. Aliquots of 5 ml were withdrawn at different time intervals up to 10 hr and a 5 ml of fresh medium was added to replace the sample that was withdrawn. Drug content of the beads was determined by UV/Visible spectroscopy at 266 nm, after suitable dilution of the samples [13-17].

f) Flow properties [18-25]

i) Bulk Density

The bulk density is defined as the mass of powder divided by bulk volume. The bulk density was calculated by dividing the weight of the samples in grams by the final volume in cm.

$$\textbf{Bulk Density} = \frac{\textbf{Mass of the micro spheres}}{\textbf{Bulk volume of the micro spheres}}$$

ii) Tapped Density

Tapped density is the volume of powder determined by tapping by using a measuring cylinder containing weighed amount of sample. The cylinder containing known amount of microspheres was tapped for about 1 minute on a tapped density apparatus until it gives constant volume.

$$\textbf{Tapped Density} = \frac{\textbf{Mass of the microspheres}}{\textbf{Tapped volume of the microspheres}}$$

iii) Carr's Compressibility Index

This is an important property in maintaining uniform weight. It is calculated using following equation**,**

$$\textbf{\% compressibility index} = 1 - \frac{\textbf{Bulk density}}{\textbf{Tapped density}} \times 100$$

iv) Hausner ratio

A similar index like percentage compressibility index has been defined by Hausner. Values less than 1.25 indicate good flow, where as greater than 1.25 indicates poor flow. Added glidant normally improve flow of the material under study. Hausner's ratio can be calculated by formula,

$$\textbf{Hausner ratio} = \frac{\textbf{Tapped density}}{\textbf{Bulk density}} \times 100$$

v) Angle of Repose (θ)

Interparticle forces between particles as well as flow characteristics of powders are evaluated by angle of repose. Angle of repose is defined as the maximum angle possible between the surface and the horizontal plane. The angle of repose of each powder blend was determined by glass funnel method. Powders were weighed accurately and passed freely through the funnel so as to form a heap. The height of funnel was so adjusted that the tip of the funnel just touched the apex of the heap. The diameter of the powder cone so formed was measured and the angle of repose was calculated using the following equation,

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

Where,
θ = angle of repose
h = height of the pile,
r = radius of the powder cone respectively.
For good flowing materials then, angle of repose should be less than 30°.

RESULTS AND DISCUSSION

Preformulation Studies

The overall objective of preformulation studies is to generate useful information to the formulator in developing stable and bioavailable dosage forms that can be mass produced.

a) Drug and excipients compatibility studies

FTIR spectra are a valuable tool to explore the possible interactions between the drug and polymer.

b) Fourier Transform Infra Red Spectroscopy (FTIR)

The spectra of the pure drug and drug with excipients are shown in the following figure

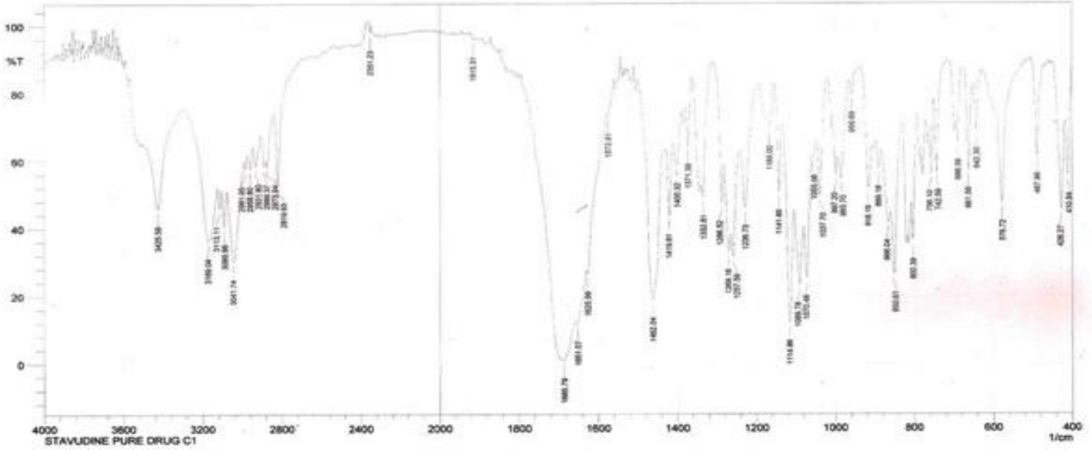

Fig-1: FTIR spectra of Stavudine

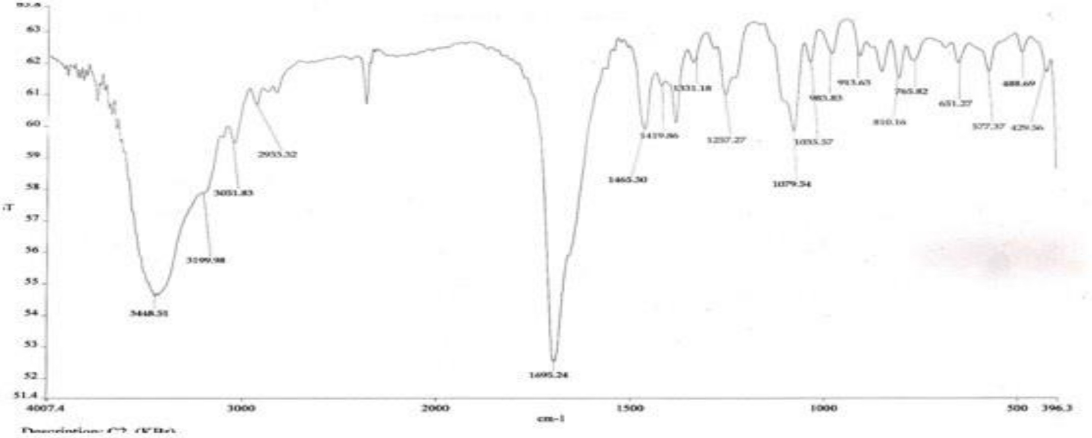

Fig-2: FTIR spectra of F1 formulation

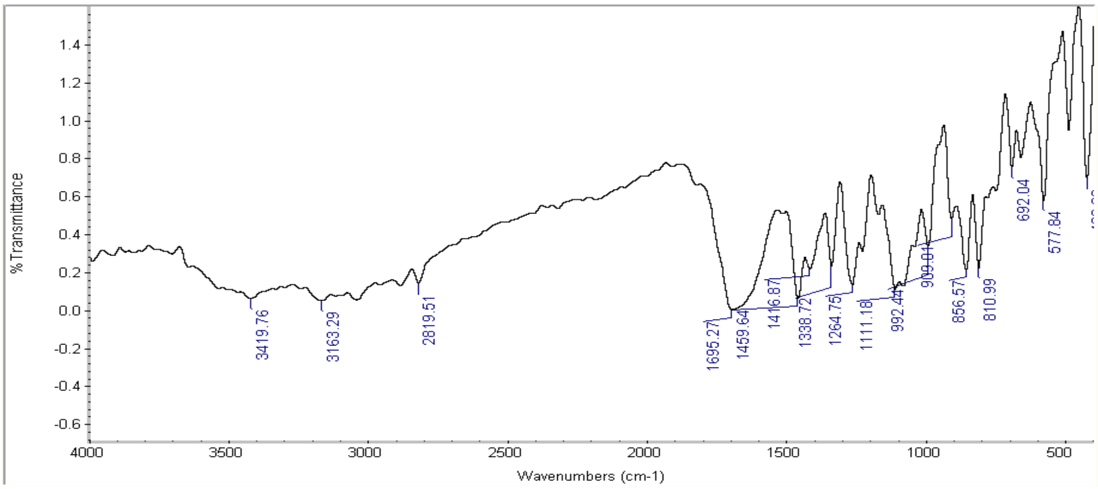

Fig-3: FTIR spectra of F2 formulation

Observation

Table 2: FTIR spectra data of Drug and floating microcarriers

S.NO	Sample	Characteristic bands(cm^{-1})	F1 formulation	F2 formulation	Possible functionalities
1	Stavudine	3425.58	3448.51	3441.97	N-H stretching
		2931.80	2933.32	2922.50	C-H stretching
		1685.79	1695.24	1691.03	C=O stretching

DISSCUSION

The IR spectra of pure Stavudine and F1, F2 formulations are showed in figure 1, 2, 3. There is no change in the nature and position of the Characteristic band for drug and drug-polymers used in the formulation, it can be concluded that there is no chemical interaction between the drug and polymer.

Standard Curve of Stavudine

Table 3: Data for standard curve of Stavudine in P^H 1.2 HCL buffer

Sno	Concentration (mcg/ml)	Absorbence
1	2	0.112
2	4	0.184
3	6	0.263
4	8	0.352
5	10	0.441

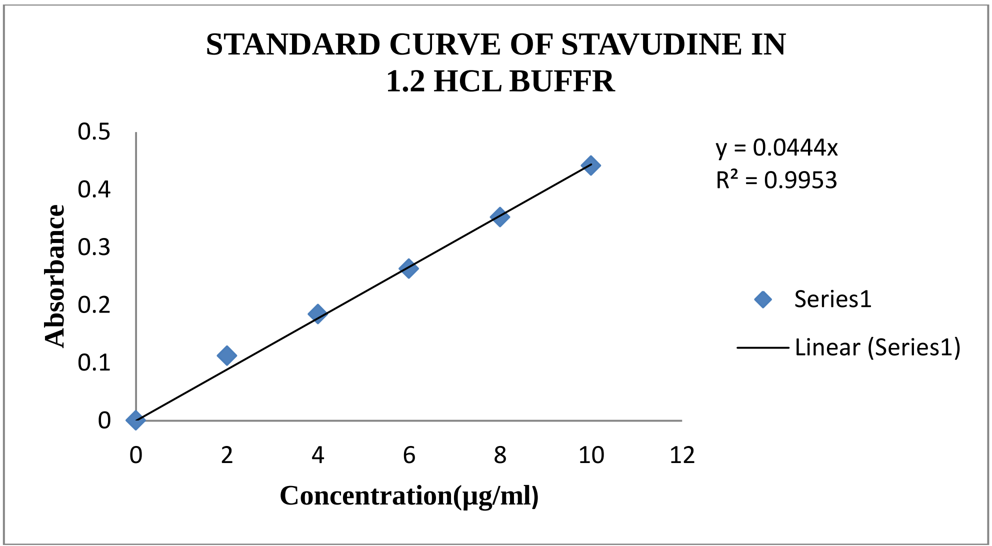

Fig-4: Standard curve of Stavudine in P^H 1.2 HCL buffer

Table 4: Data for standard curve of Stavudine in P^H 7.4 Phosphate buffer

Sno	Concentration (mcg/ml)	Absorbence
1	2	0.076
2	4	0.165
3	6	0.255
4	8	0.344
5	10	0.415

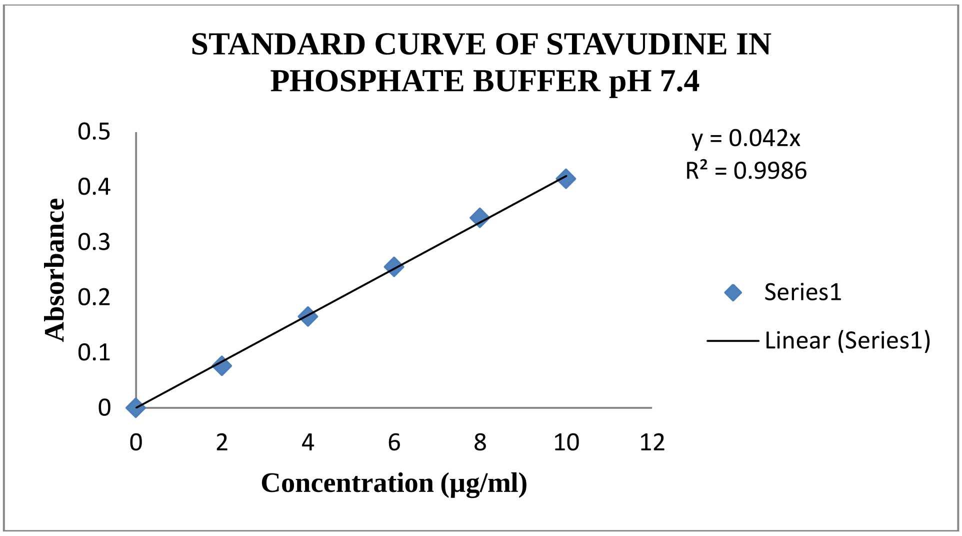

Fig-5: Standard curve of Stavudine in P^H 7.4 Phosphate buffer

Floating Behaviour

100% buoyancy was observed for all the formulations. Liquid paraffin has lower relative density (0.86). It helped the microcarriers to become buoyant. There was no lag time was observed, the microcarriers immediately floated and remained floating for 10hrs. The floating behavior was depending on the amount of the liquid paraffin entrapped in the microcarriers.

Table 5: Floating behavior of floating microcarriers

Sno	Formulation	% Buoyancy
1	F1	100%
2	F2	100%
3	F3	100%
4	F4	100%
5	F5	100%
6	F6	100%

Flow Propertis

Table 6: Flow properties of floating microcarriers

S.NO	Formulation	Angle of repose	Carr's index	Hausner's ratio
1	F1	20.56 ±0.92	14.46 ± 0.91	1.17 ± 0.03
2	F2	21.54 ± 1.25	16.42 ± 0.97	1.14 ± 0.03
3	F3	21.79 ± 0.72	15.58± 1.29	1.21 ± 0.03
4	F4	21.96 ± 0.97	13.08 ± 0.29	1.14 ± 0.03
5	F5	22.46 ± 1.02	15.56 ± 0.97	1.15 ± 0.01
6	F6	22.86 ± 0.7	13.31 ±0.35	1.16 ± 0.02

DISCUSSION

In our study, three flow measurement types were employed; the angle of repose, Carr's index (compressibility index), and Hausner's ratio and their results are tabulated in Table 6. The angle of repose (ɵ) is a characteristic of the internal friction or cohesion of the particles, the value of the angle of repose will be high if the powder is cohesive and low if the powder is non-cohesive. The prepared floating microcarriers showed ɵ values in 20-22°.

Carr's index up to 21 is considered of acceptable flow properties. Hausner ratio was related to the inter particle friction, the powders with low interparticle friction, had ratios of approximately 1.25 indicating good flow. The prepared floating microcarriers showed good flowing properties.

Particle Size

Particle size was determined by using optical microscopy. The mean particle size was in range of 0.59 mm to 1.254 mm.

Table 7: The mean particle size of F1 formulation

Sno	Particle Size Range (µm)	Mean Size µm (d)	Number Of Particles (n)	n×d
1	400-450	425	10	4250
2	451-500	475	10	4750
3	501-550	525	11	5775
4	551-600	575	10	5750
5	601-650	625	2	1250
6	651-700	675	7	4725
			Σn=50	Σnd=2950

Mean particle size = $\frac{\Sigma nd}{\Sigma n}$ = 590µm = 0.59mm

Table 8: The mean particle size of F2 formulation

SNO	Particle Size Range (µm)	Mean Size µm (d)	Number Of Particles (n)	n×d
1	650-700	675	7	4725
2	701-750	725	10	7250
3	751-800	775	12	9300
4	801-850	825	0	0
5	851-900	875	6	5250
6	901-950	925	6	5550
7	951-1000	975	9	8775
			Σn=50	Σnd=40850

Mean particle size = $\frac{\Sigma nd}{\Sigma n}$ = 825µm = 0.825mm

Table 9: The mean particle size of F3 formulation

SNO	Particle Size Range (µm)	Mean Size µm (d)	Number Of Particles (n)	n×d
1	850-900	875	4	3500
2	901-950	925	4	3700
3	951-1000	975	11	10725
4	1001-1050	1025	11	11275
5	1051-1100	1075	8	8600
6	1101-1150	1125	9	10125
			Σn=50	Σnd=47925

Mean particle size = $\frac{\Sigma nd}{\Sigma n}$ = 958.5µm = 0.9585mm

Table 10: The mean particle size of F4 formulation

SNO	Particle Size Range (µm)	Mean Size µm (d)	Number Of Particles (n)	n×d
1	950-1000	975	5	4875
2	1001-1050	1025	8	8200
3	1051-1100	1075	11	4825
4	1101-1150	1125	12	13500
5	1151-1200	1175	7	8225
6	1201-1250	1225	7	8575
			ΣN=50	Σnd=55200

Mean particle size = $\frac{\Sigma nd}{\Sigma n}$ = 1104µm =1.104mm

Table 11: The mean particle size of F5 formulation

SNO	Particle Size Range (μm)	Mean Size μm (d)	Number Of Particles (n)	n×d
1	1050-1100	1075	8	8600
2	1101-1150	1125	6	6750
3	1151-1200	1175	13	15275
4	1201-1250	1225	10	12250
5	1251-1300	1275	7	8925
6	1301-1350	1325	6	7950
			Σn=50	Σnd=59750

$$\text{Mean particle size} = \frac{\Sigma nd}{\Sigma n} = 1195\mu m = 1.195mm$$

Table 12: The mean particle size of F6 formulation

SNO	Particle Size Range (μm)	Mean Size μm (d)	Number Of Particles (n)	n×d
1	1100-1150	1125	4	4500
2	1151-1200	1175	5	5875
3	1201-1250	1225	13	15925
4	1251-1300	1275	17	21675
5	1301-1350	1325	8	10600
6	1351-1400	1375	3	4125
			Σn=50	Σnd=62700

$$\text{Mean particle size} = \frac{\Sigma nd}{\Sigma n} = 1254\mu m = 1.254mm$$

Table 13: The mean particle size of the floating microcarriers

SNO	Formulation	Mean Particle Size (mm)
1	F1	0.59
2	F2	0.825
3	F3	0.9585
4	F4	1.04
5	F5	1.195
6	F6	1.25

DISCUSSION

The mean particle size of the floating microcarriers was increased as the concentration of oil increases. It suggests that the as the concentration of oil increases the amount of oil entrapped in floating microcarriers was increased.

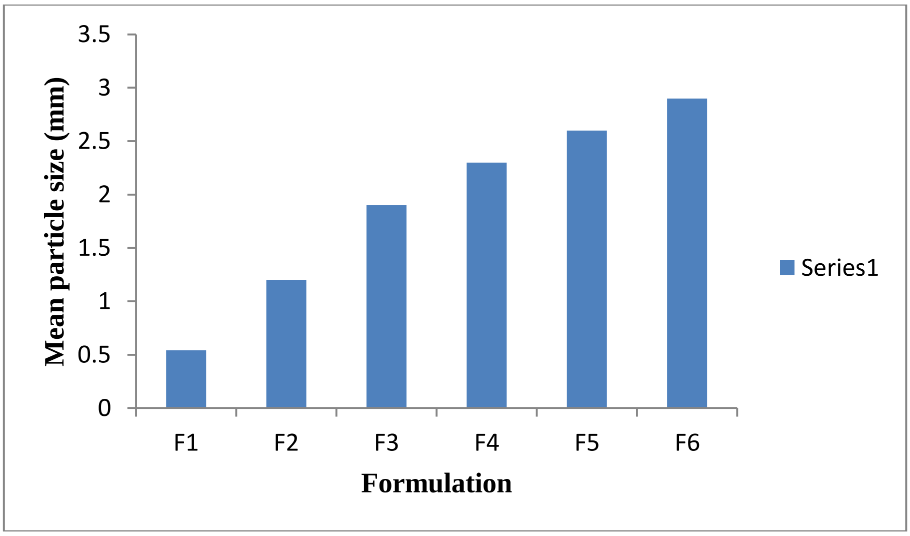

Fig-6: The mean particle size of Floating microcarriers

Drug Entrapment Efficiency

The drug entrapment efficiency was determined by UV spectrophotometric method.

Table 14: The drug entrapment efficiency

SNO	Formulation	Absorbance	% Drug entapment efficiency
1	1	0.307	69.61
2	2	0.283	64.17
3	3	0.258	58.50
4	4	0.201	45.57
5	5	0.244	55.32
6	6	0.197	44.6

DISCUSSION

The %drug entrapment was found in the range of 44.60 to 69.61. F1 formulation showed highest entrapment efficiency (69.91). Up on addition of oil to the formulation the %drug entrapment efficiency was decreased, a gradual decrease in the %drug entrapment efficiency was observed as the concentration of the oil increases due to enhanced volume oil occupied the most of the volume of a single microcarrier and prevented the entrapment of sufficient amount of drug.

In-Vitro Drug Release

Table 15: Percentage drug release of F1 formulation:

SNO	Time (hr)	Absorbance	% Drug release
1	1	0.051	12.281
2	2	0.096	25.866
3	3	0.121	33.519
4	4	0.143	40.312
5	5	0.176	50.446
6	6	0.204	59.133
7	7	0.241	70.570

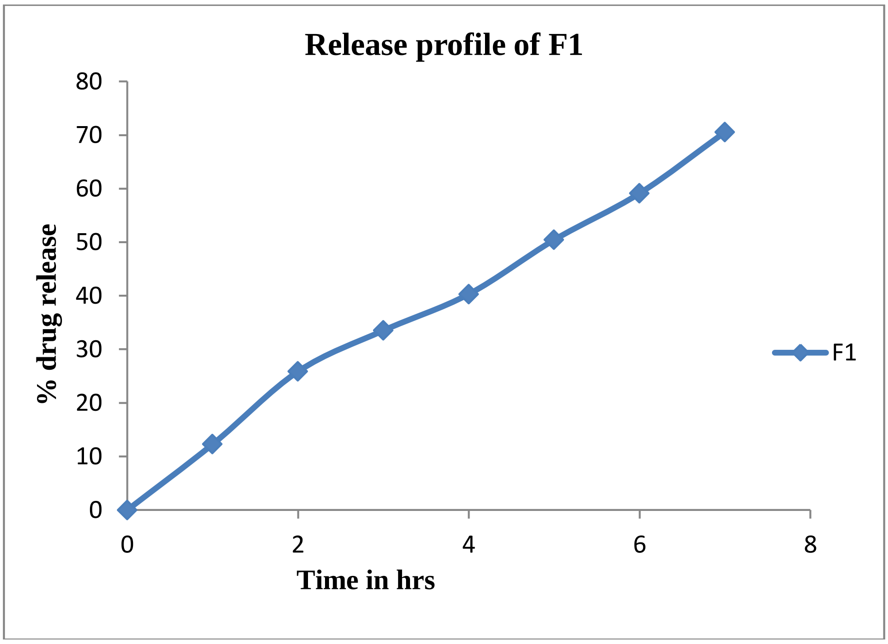

Fig-7: %Drug release profile of F1 formulation

Table 16: Percentage drug release of F2 formulation:

SNO	Time (hr)	Absorbance	%Drug release
1	1	0.041	10.064
2	2	0.068	18.917
3	3	0.097	28.471
4	4	0.119	35.797
5	5	0.141	43.162
6	6	0.173	53.826
7	7	0.199	62.593

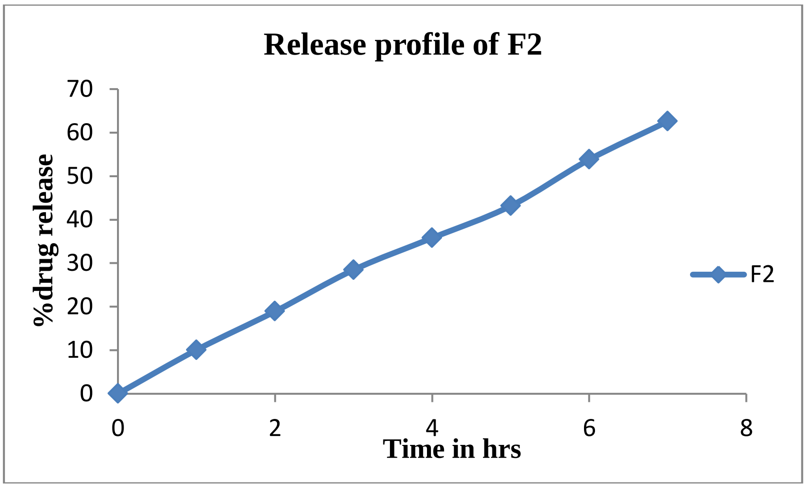

Fig-8: %Drug release profile of F2 formulation

Table 17: Percentage drug release of F3 formulation:

S.No	Time (hr)	Absorbance	%Drug release
1	1	0.049	13.898
2	2	0.061	18.265
3	3	0.107	28.373
4	4	0.125	34.963
5	5	0.164	41.589
6	6	0.164	55.757
7	7	0.195	67.142

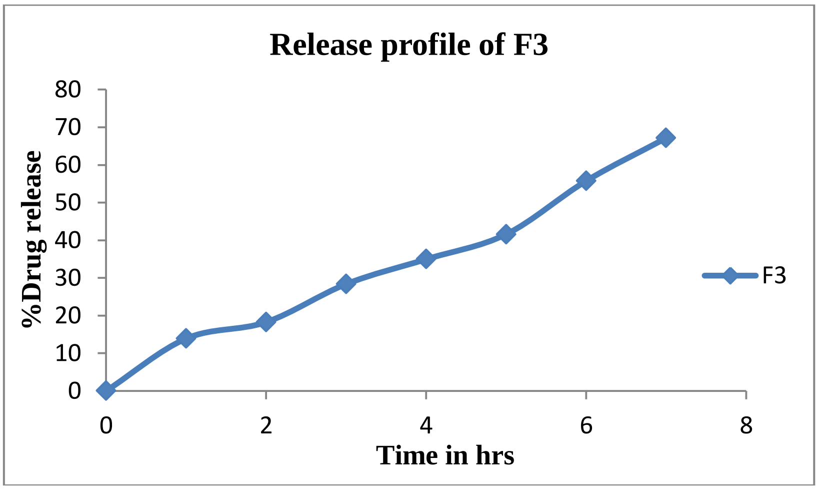

Fig-9: %Drug release profile of F3 formulation

Table 18: Percentage drug release of F4 formulation:

S.No	Time (hr)	Absorbance	%Drug release
1	1	0.032	10.042
2	2	0.041	14.227
3	3	0.064	24.859
4	4	0.081	32.796
5	5	0.099	41.236
6	6	0.111	46.969
7	7	0.129	55.985

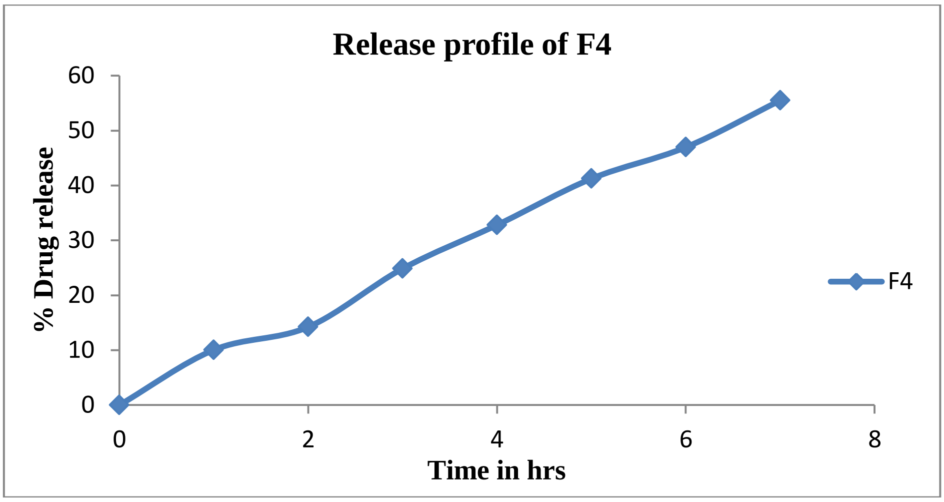

Fig-10: %Drug release profile of F4 formulation

Table 19: Percentage drug release of F5 formulation

S.No	Time (hr)	Absorbance	%Drug release
1	1	0.037	10.162
2	2	0.050	15.132
3	3	0.068	22.019
4	4	0.097	33.101
5	5	0.118	41.221
6	6	0.131	46.361
7	7	0.159	57.198

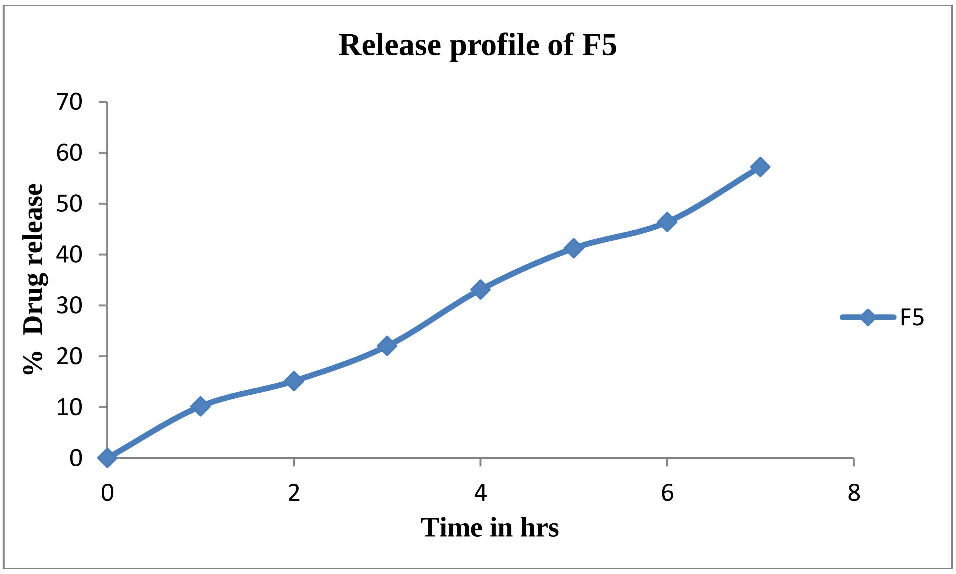

Fig-11: %Drug release profile of F5 formulation

Table 20: Percentage drug release of F6 formulation:

S.No	Time (hr)	Absorbance	%Drug release
1	1	0.032	10.260
2	2	0.043	15.005
3	3	0.057	22.120
4	4	0.082	33.963
5	5	0.099	42.120
6	6	0.109	47.039
7	7	0.121	52.923

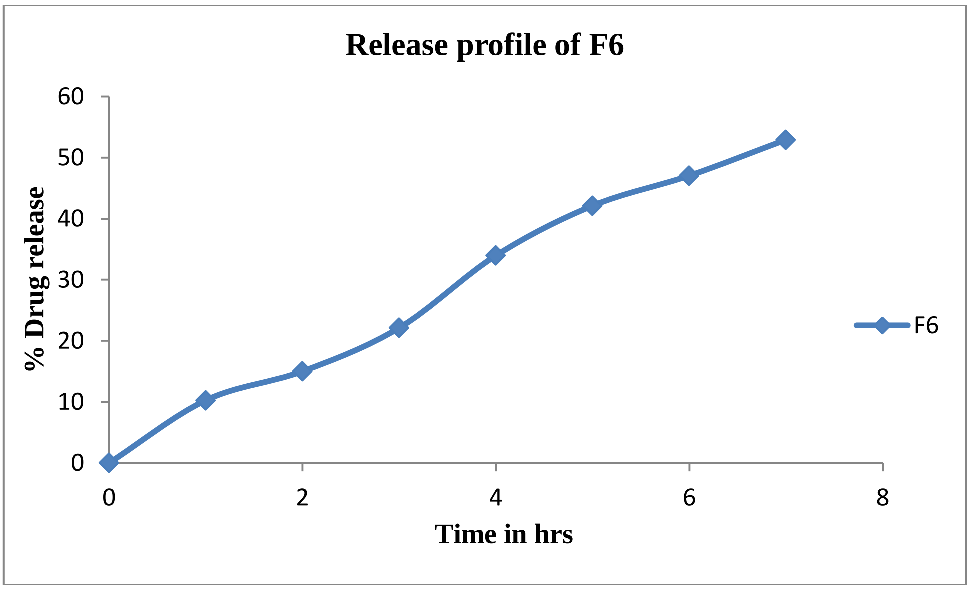

Fig-12: %Drug release profile of F6 formulation

Table 21: In-vitro drug release of floating microcarriers

Time(hr)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	12.281	10.064	13.898	10.042	10.162	10.260
2	25.866	18.917	17.265	15.227	15.132	15.005
3	33.519	28.471	26.373	24.859	22.019	22.120
4	40.312	35.797	33.963	32.796	33.101	29.963
5	50.446	43.162	40.589	38.236	41.221	36.120
6	59.133	53.826	49.757	46.969	58.361	43.039
7	70.570	62.593	60.142	55.485	67.198	51.923
8	78.302	71.917	68.260	64.312	76.214	59.757
9	89.231	82.132	77.227	72.281	84.898	67.198
10	97.864	94.265	89.917	80.064	95.260	75.132

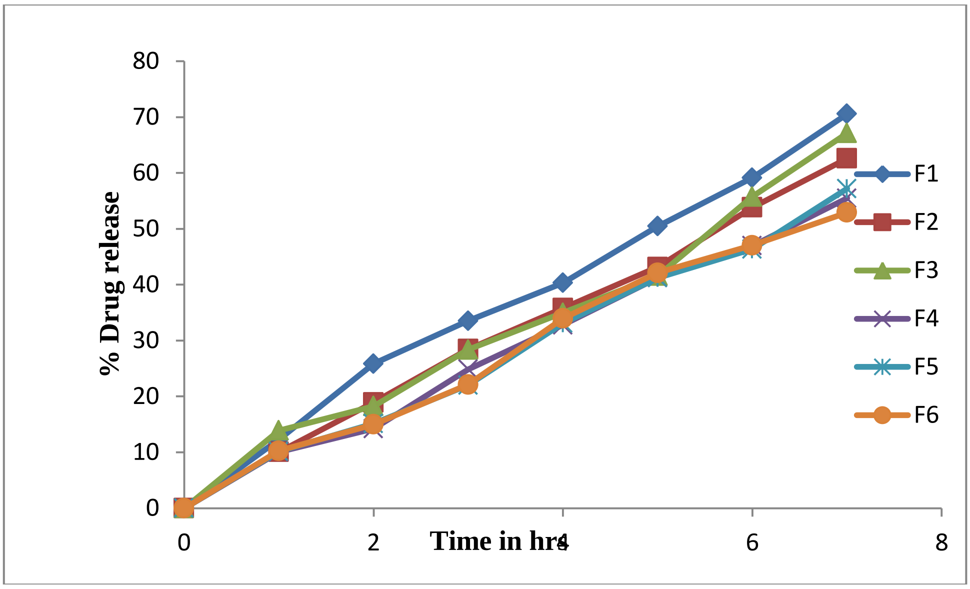

Fig-13: %Drug release profiles of Floating microcarriers

DISCUSSION

The amount of the liquid paraffin in the formulation played a vital role in the drug release. As the concentration of the liquid paraffin increases the dug release was prolonged for more than 7hrs. The drug release was extended to more than 7hrs.The low concentration of oil containing formulation exhibited greater release of drug. As the concentration of oil increases, the drug release decreased to certain extent, it implies that the use of different concentration of permit efficient control of the release of the drug.

SUMMARY AND CONCLUSION

The present work showed that the emulsion ionotopic gelation technique can be effectively used to prepare floating microcarriers. The floating microcarriers successfully deliver the drug for a prolong duration of time. The drug release can be controlled by varying the concentration of the oil amount. The drugs having shorter biological half life can successfully deliver by using floating drug delivery system for longer period of time and also increase the bioavailability of those drugs having good absorption in the upper part of GI Tract.

REFERENCES

1. Mehul, R. T., Wilson, B., Shanaz, B., & Bincy, R. (2011). Formulation and in vitro evaluation of floating microspheres of anti-retro viral drug as a gastro retentive dosage form. *International Journal of Research in Pharmacy and Chemistry (IJRPC)*, *1*(3).
2. Nayak, A. K., Malakar, J., & Sen, K. K. (2010). Gastroretentive drug delivery technologies: Current approaches and future potential. *Journal of Pharmaceutical Education and Research*, *1*(2), 1.
3. Zhang, X., Lee, I., & Berdis, A. J. (2005). A potential chemotherapeutic strategy for the selective inhibition of promutagenic DNA synthesis by nonnatural nucleotides. *Biochemistry*, *44*(39), 13111-13121.
4. Kumar, D., Dave, V., Lewis, S., Parmar, B., Gajbhiye, K. R., & Paliwal, S. (2010). Design and evaluation of sustained-release matrix once daily formulation of stavudine. *International journal of drug delivery*, *2*(2).
5. Sahoo, S. K., Mallick, A. A., Barik, B. B., & Senapati, P. C. (2005). Formulation and in vitro Evaluation of Eudragit® Microspheres of Stavudine. *Tropical Journal of Pharmaceutical Research*, *4*(1), 369-375.
6. Tanvi, R., & Anupama, D. (2011). Novel polymeric combinations for gastroretentive microspheres of stavudine. *International Journal of Drug Development and Research*.
7. Karthikeyan, D., Karthikeyan, M., & Ramasamy, C. (2010). Development of floating microspheres to improve oral bioavalibity of cefpodoxime proxetil. *Acta Pharmaceutica Sciencia*, *52*, 101-104.
8. Khan, A. D., & Bajpai, M. (2011). Formulation and Evaluation of Floating beads of Verapamil hydrochloride. *International Journal of PharmTech Research*, *3*(3), 1537-1546.
9. Salunke, P., Rane, B., Bakliwal, S., & Pawar, S. (2010). Floating microcarriers of an antidiabetic drug: Preparation and its in-vitro evaluation. *J. Pharm. Sci. Technol*, *2*, 230-240.
10. Fursule, R. A., Patra, C. H. N., Patil, G. B., Kosalge, S. B., Patil, P. O., & Deshmukh, P. K. (2009). Study of multiparticulate floating drug delivery system prepared by emulsion gelation

technique. *International journal of chemtech research*, *1*(2), 162-167.

11. Jaiswal, D., Bhattacharya, A., Yadav, I. K., Singh, H. P., Chandra, D., & Jain, D. A. (2009). Formulation and evaluation of oil entrapped floating alginate beads of ranitidine hydrochloride. *Int J Pharm Pharm Sci*, *1*(3), 128-40.
12. Chawla, G., & Bansal, A. (2003). A means to address regional variability in intestinal drug absorption. *Pharm Technol*, *27*(6), 50-68.
13. Vyas, S. P., & Khar, R. K. (2004). *Targeted & controlled drug delivery: Novel carrier systems*. CBS publishers & distributors.
14. Streubel, A., Siepmann, J., & Bodmeier, R. (2006). Gastroretentive drug delivery systems. *Expert opinion on drug delivery*, *3*(2), 217-233.
15. Iannuccelli, V., Coppi, G., Bernabei, M. T., & Cameroni, R. (1998). Air compartment multiple-unit system for prolonged gastric residence. Part I. Formulation study. *International journal of pharmaceutics*, *174*(1), 47-54.
16. Garg, R., & Gupta, G. D. (2008). Progress in controlled gastroretentive delivery systems. *Tropical Journal of Pharmaceutical Research*, *7*(3), 1055-1066.
17. Rouge, N., Allémann, E., Gex-Fabry, M., Balant, L., Cole, E. T., Buri, P., & Doelker, E. (1998). Comparative pharmacokinetic study of a floating multiple-unit capsule, a high-density multiple-unit capsule and an immediate-release tablet containing 25 mg atenolol. *Pharmaceutica Acta Helvetiae*, *73*(2), 81-87.
18. Streubel, A., Siepmann, J., & Bodmeier, R. (2003). Multiple unit gastroretentive drug delivery systems: a new preparation method for low density microparticles. *Journal of microencapsulation*, *20*(3), 329-347.
19. Goole, J., Vanderbist, F., & Amighi, K. (2007). Development and evaluation of new multiple-unit levodopa sustained-release floating dosage forms. *International journal of pharmaceutics*, *334*(1), 35-41.
20. Sharma, S., & Pawar, A. (2006). Low density multiparticulate system for pulsatile release of meloxicam. *International journal of pharmaceutics*, *313*(1), 150-158.
21. Santus, G., Lazzarini, C., Bottoni, G., Sandefer, E. P., Page, R. C., Doll, W. J., ... & Digenis, G. A. (1997). An in vitro-in vivo investigation of oral bioadhesive controlled release furosemide formulations. *European journal of pharmaceutics and biopharmaceutics*, *44*(1), 39-52.
22. Klausner, E. A., Lavy, E., Friedman, M., & Hoffman, A. (2003). Expandable gastroretentive dosage forms. *Journal of controlled release*, *90*(2), 143-162.
23. Deshpande, A. A., Shah, N. H., Rhodes, C. T., & Malick, W. (1997). Development of a novel controlled-release system for gastric retention. *Pharmaceutical research*, *14*(6), 815-819.
24. Park, K. (1988). Enzyme-digestible swelling hydrogels as platforms for long-term oral drug delivery: synthesis and characterization. *Biomaterials*, *9*(5), 435-441.
25. Fujimori, J., Machida, Y., & Nagai, T. (1994). Preparation of a magnetically-responsive tablet and confirmation of its gastric residence in beagle dogs. *STP Pharma sciences*, *4*(6), 425-430.