Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 13 [4] March 2024: 17-23

Online ISSN 2277-1808
Journal's URL: http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Optimization, Development and Characterization of Clopidogrel Bisulphate Microcapsules by DoE

S. Ramya Krishna*, B. Sowjanya Lakshmi, Y. Sailaja, A. Ramu, S. Vidyadhara.
Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chowdavaram, Guntur, A.P., India.
Email id: ramya.krishna.seelam@gmail.com

ABSTRACT

Gastric emptying is one of the complex processes and therefore the efficiency of in vivo drug delivery can be highly variable. Extending the residence time in the stomach beyond 12 hours has a significant impact on drugs that have an absorption window in the stomach and large intestine. Microparticle drug delivery is an important oral drug delivery method that is more effective than single-dose tablets. An existing method to increase drug retention time in the stomach by delivering multiparticulate drug is to create floating microcapsules. They increase the bioavailability of the drug by increasing the rate of drug absorption and reducing the risk of local irritation. In the current research study, ethylcellulose 7 cps was used as the drug release polymer and HPMC 5 cps as the pore former to formulate the BCS class II drug clopidogrel into controlled-release microcapsules to improve the gastric retention of the drug. A full 3^2 factorial design was chosen to optimize the formulation of clopidogrel microcapsules using solvent evaporation technology. The maximum drug encapsulation efficiency in the F optima-2 formulation was 88.58%. The F optima-3 formulation has a small particle size of 278.98 µm, and flotation studies show that all formulations have good buoyancy. The highest microcapsule yield was found in the F optima-2 formulation with 79.45% efficiency. All formulations were subjected to in vitro drug release studies, and the Peppas n value of all formulations was above 0.5; This indicates that the drug release mechanism is non-Fickian diffusion.

Keywords: Full 3^2 factorial design, Clopidogrel, Gastric transit time, Gastric emptying, Microcapsules.

Received 16.01.2024 Revised 26.02.2024 Accepted 19.03.2024

INTRODUCTION

In the literature, the ease of oral administration has attracted the attention of different drugs, but changes in the gastrointestinal physiology during the change period have no effect on bioavailability and irreversible effects. Most drugs are well absorbed throughout the gastrointestinal tract, but some drugs are poorly absorbed in the large intestine and have a suitable location only in the stomach. These drugs interfere with the digestive system when produced in large quantities. Improving intestinal residence times should also be considered in the development of these drugs [1]. One way to improve stomach residence time is to mix the drug with a floating material that is less potent than gastric juice [2].

Even the distribution of several substances in the stomach can provide greater absorption and reduce the risk of local irritation compared to a single dosage form. Long-term dependence of the stomach is to control not only the time but also the place in the stomach, ensuring that the body is sent to a stable place for proper delivery of the drug.

Clopidogrel bisulfate belongs to the thienopyridine class of antiplatelet drugs. It is used to reduce the formation of blood clots in patients at risk of heart attack and stroke. It is a BCS-II drug with low solubility and high permeability. Therefore, controlled release formulations of clopidogrel help increase its bioavailability [3-6]. Clopidogrel bisulfate is an acidic pKa drug whose absorption depends on pH, and being an acidic drug, it is better absorbed in the stomach and less absorbed in the intestine. When formulated as a regular controlled release dosage form, it passes through the stomach and intestines due to intestinal obstruction and therefore cannot control plasma levels as intestinal absorption is reduced [7]. Therefore, microencapsulating clopidogrel helps to increase the residence time in the stomach and thus maintain a constant plasma concentration. In this study, clopidogrel was formulated into floating microcapsules to improve the gastric residence time and hence bioavailability of bisulfate.

MATERIAL AND METHODS

Pure Clopidogrel Hydrogen Sulphate was received as a gift from Emco Industries, Hyderabad. Ethylcellulose and HPMC were obtained from Dow Chemicals. DCM, IPA, Tween 80, HCl and sulfuric acid were purchased from SD Fine Chemicals Ltd, Mumbai.

Drug-Excipient Compatibility Studies

FT-IR Spectroscopy: The physicochemical compatibility of clopidogrel and methylcellulose used in this study was determined by infrared spectroscopy studies using a Bruker Fourier transform infrared spectrophotometer. The sample was prepared by mixing 100 mg of the drug used in the preparation of floating microcapsules with 100 mg of ethylcellulose. The samples were analyzed in the diffuse reflection spectra, graphs were drawn with the KBR particle method, and spectra were recorded in the wavelength range of 4000 cm^{-1} to 400 cm^{-1}. The spectra obtained for pure substances are compared with those of physical mixtures of substances and polymers.

Preparation of Floating Microcapsules

Experimental design

A full 3^2 factorial design has been used to optimize clopidogrel-loaded EC 7cps + HPMC 5cps microcapsules using the solvent evaporation technique [14,15] (Table 1). In this study, encapsulation efficiency was considered as a measurable parameter. To examine the effects of three factors (clopidogrel concentration (A), EC 7cps concentration (B), HPMC 5cps concentration (C)), a design matrix of 9 experiments was created using DOE Pro XL software. Regarding response variables, for example, the percentage of drug released at 1 hour (D1) and the percentage of drug released at 8 hours (D8) were measurably calculated. Weight volume (50 ml), IPA and DCM ratio (1:1), water phase volume (500 ml), Tween concentration (2.5 ml), stirring speed (500 rpm) and temperature (ambient) were maintained.

Method of preparation of floating microcapsules [7-9]

Clopidogrel floating microcapsules were prepared by the solvent evaporation technique described previously. Dissolve clopidogrel, EC and HPMC in 0.5 ml of a heavy mixture of concentrated acidified IPA and DCM. Fill the solution into a syringe fitted with a 22-gauge needle. It was placed in a pot containing 500 ml of water and 2.5 ml of Tween as a continuous phase at a rate of 50 drops/min. The continuous phase was mixed at 500 rpm using a 3-blade Remi mixer. After the addition of the drug-polymer solution was completed, the mixture was stirred for 2 hours. Microcapsules floating on the section were collected and dried in an oven at 400°C for 1 hour. Place the prepared floating microcapsules in airtight containers for further testing. The dried microcapsules were weighed and the results were determined. The composition of the various formulations is shown in Table 1.

Table 1: Full factorial DOE and Compositions of Clopidogrel Floating Microcapsules

S no:	INGREDIENTS	LL	LM	LH	ML	MM	MH	HL	HM	HH
		F1	F2	F3	F4	F5	F6	F7	F8	F9
1	CLOPIDOGREL	100	100	100	100	100	100	100	100	100
2	EC	50	50	50	100	100	100	200	200	200
3	HPMC	0.5	1	2	0.5	1	2	0.5	1	2

*EC 7cps-L-50mg, H- 200mg, *HPMC 5cps- L- 0.4mg, H- 4mg.

Characterization of Microcapsules [10-15]

Particle size and size distribution:

The size of floating microcapsules was measured using an optical microscope, and the average microcapsule size was calculated by measuring 100 particles with the help of a calibrated eyepiece micrometer.

Surface morphology:

Scanning electron microscopy was used to determine distribution, surface area, texture, and examine the morphology of the fracture or segment. SEM studies were performed using a JEOL JSMT-330A scanning microscope (Japan). SEM samples were prepared by lightly sprinkling microcapsule powder on double-sided tape attached to an aluminum rod. The stud is then gold plated using a sputtering machine to a thickness of approximately 300 Angstroms. Micrographs were taken with the help of SEM analyzer.

Yield (%):

The percentage of floating microcapsules is calculated by dividing the weight of the product by the total non-volatile material used to prepare the floating microcapsules and is expressed by the following formula.

$$\% \, \boldsymbol{yield} = \frac{\textbf{Actual weight of product}}{\textbf{Total weight of drug and excipients}} \times \mathbf{100}$$

Drug entrapment efficiency and Percentage Loading Efficiency:

Determine the amount of drug encapsulated by crushing 100 mg drug equivalent microcapsules in a 100 ml beaker containing 50 ml of 0.1N HCl. Place the beaker on the magnetic stirrer and adjust the speed accordingly. After 3 h, samples were removed and absorbance was measured at 240 nm using a thermal UV spectrophotometer using 0.1 N HCl as blank. The encapsulation percentage and loading efficiency percentage are calculated as follows:

$$DEE = \frac{\textbf{Amount of drug actually present}}{\textbf{Amount of drug taken}} \times 100$$

Floating characterization:

Place 100 floating microcapsules of each formulation in a 100 ml beaker containing 70 ml of water and leave for 24 hours. Observe the number of microcapsules floating on the surface after 24 hours.

$$\%\ Buoyancy = \frac{No.of\ floated\ \ microcapsules\ after\ 24hrs}{No.of\ floating\ microcapsules\ taken} \times 100$$

Drug release studies [16-19]

Microcapsules were packaged into hard gelatin capsule shells, and *in vitro* dissolution studies were performed in a USP type I dissolution tester. Samples are taken at 0, 0.5 and 1, 2, 4 and 8 hours. After appropriate dilution, use a UV-visible spectrophotometer to analyze at a wavelength of 240 nm.

Drug release kinetic studies:

Fit data from *in vitro* release studies to various kinetic equations to determine the mechanism of drug release. The kinetic models used are:

$$Qt = K_0\ t \text{ (zero-order equation)}$$
$$\ln Q_t = \ln Q_0 - K_1\ t \text{ (first-order equation)}$$
$$Q_t = K_h\ t_{1/2} \text{ (Higuchi equation)}$$

Where Q_t is the amount of drug release in time t, Q_0 is the initial amount of drug in the microsphere, and K_0, K_1, and Kh are rate constants of zero order, first order and Higuchi equations respectively. Further to confirm the mechanism of drug release, the first 60% of drug release was fitted in Korsemeyer-Peppas model.

$$M_t / M_\infty = k\ tn$$

where M_t is the amount of drug release at time t and $M_\infty$is the amount release at time t=∞, thus $M_t / M_\infty$is the fraction of drug released at time t, k is the kinetic constant, and n is the diffusion exponent which can be used to characterize both mechanism for both solvent penetration and drug release.

Accelerated Stability Study:

As with all drug dosage forms, it is important to determine the stability of the dosage form. This will include storage at cold temperatures and the necessary evaluation to ensure that the product is absorbed at the same rate over its shelf life as when it was originally prepared. The development of safety studies for pharmaceutical products should be based on an understanding of the behavior and properties of drugs and safety studies of drugs. The specifications consist of the names of the tests, references to the evaluation process and approved certification standards, including the concept of different certification standards for release and shelf life specifications described in the ICH Guidelines.

RESULTS AND DISCUSSION

Drug – excipient compatibility studies

Investigating the compatability between chemicals and polymers with infrared spectroscopy. The IR spectra of pure clopidogrel and the physical mixture of drug and polymer are shown in figures 1 and 2. Since the same major peak is observed in all cases, the absence of interference effects of chemicals and polymers (alone and in combination) is noticed.

Fig 1: FT – IR spectra of Clopidogrel bisulfate

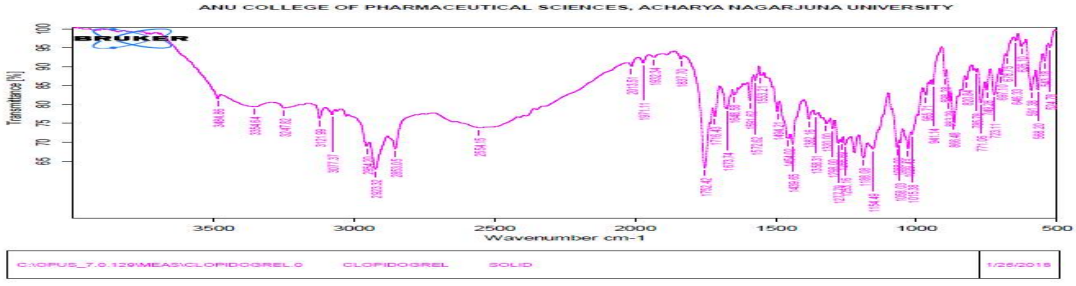


Fig 2: FT – IR spectra of Clopidogrel bisulfate and Ethyl cellulose physical mixture

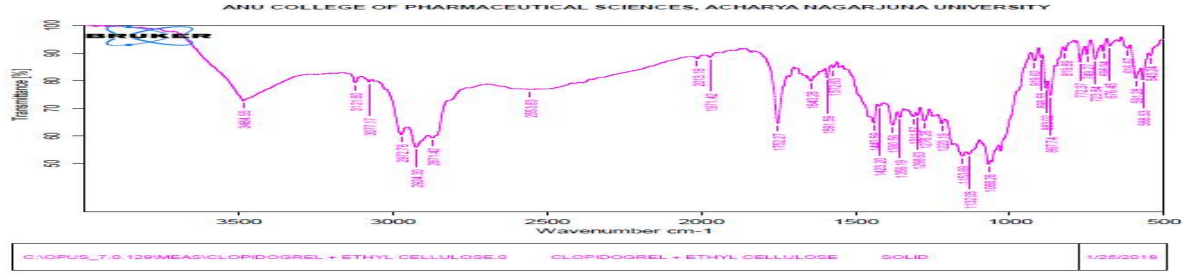


Table 2: Results of some physical characterization studies of floating microcapsules

S. No.	Formulation	Particle size (µm)	Entrapment efficiency (%)	Yield (%)
1	F1	355.86	80.39	87
2	F2	344.86	75.58	89.8
3	F3	278.98	88	90.5
4	F4	330.77	78.61	92.4
5	F5	357.29	79.97	88.7
6	F6	288.86	80.41	94.1
7	F7	386.55	85.27	95.2
8	F8	289.26	88.7	88.8
9	F9	295.32	85.6	90.8

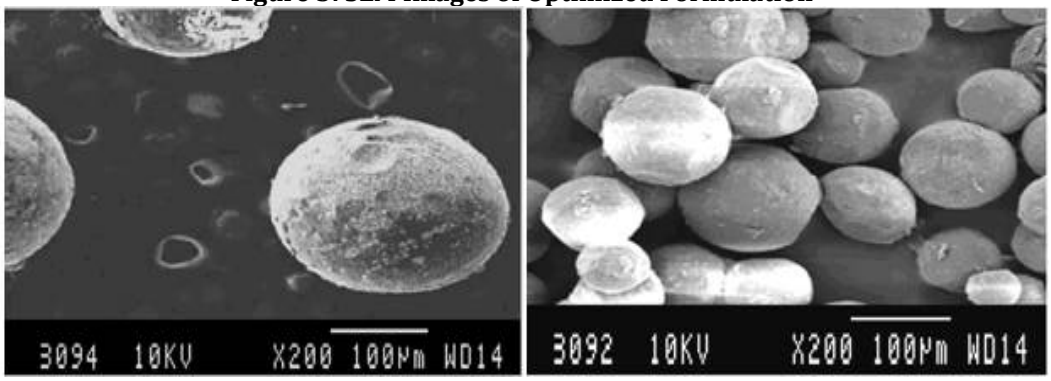
Drug entrapment efficiency and Percentage loading efficiency

The drug encapsulation efficiency of all formulations was found to be higher than 72%, indicating that the drug encapsulation efficiency in the polymer matrix is much better, as shown in Table 3. The highest drug encapsulation efficiency is F7, F8 and F9 formulations with drug encapsulation rate higher than 85%.

Shape and surface morphology

The surface morphology and internal cross-sectional structure of the floating microcapsules were examined by scanning electron microscopy. SEM micrographs of empty microcapsules and optimized formulations are shown. Microcapsules are smooth, spherical and discrete particles. The observation of small amounts of the drug on the surface of the microcapsules indicates an uneven distribution of the drug in the polymer network.

Figure 3: SEM Images of Optimized Formulation



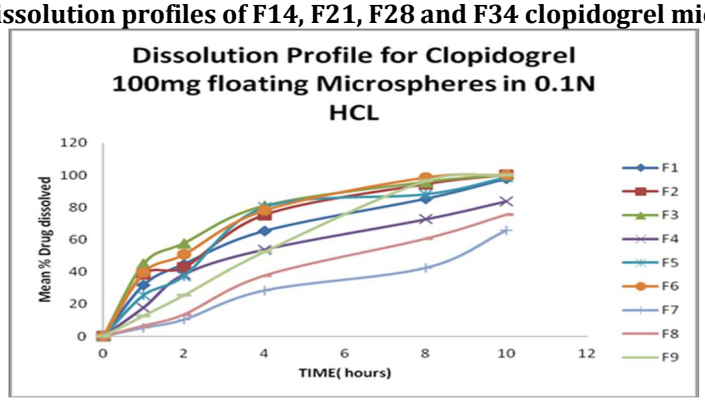
Drug release studies

The quantity and amount of drug released depends on the ethylcellulose ratio of the drug and the pore-forming agent concentration. For formulations with a 1:1 drug/polymer ratio (F1, F2, F7, F8), release depends on the HPMC concentration (the higher the HPMC level, the faster the release). For formulations with a drug/polymer ratio of 1:4, the release of F3 and F4 is very slow (<70% in 8 hours). Very rapid drug release (>80% in 2 hours) for formulation F5 and F6 with 4:1 chemical/polymer ratio is observed.

Table 3: Drug release studies of clopidogrel microcapsules

Time	Dissolution Profile For Clopidogrel Bisulphate Bisulphate Floating Microcapsules								
	LL	LM	LH	ML	MM	MH	HL	HM	HH
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	31.87	38.45	44.98	17.89	25.61	39.45	5.29	6.28	12.76
2	44.79	42.67	57.78	38.44	37.45	50.76	10.35	13.49	25.48
4	65.32	75.34	80.97	53.87	80.26	78.12	28.44	37.7	52.56
8	85.29	94.35	95.67	72.64	88.23	98.5	42.48	60.58	96.57
10	97.67	100	100	83.78	98.67	100	65.78	75.48	100

Figure 4: Dissolution profiles of F14, F21, F28 and F34 clopidogrel microcapsules



Drug release kinetic studies

Formulations F1 and F3 showed higher r values in the Korsmeyer and Peppas release plots. This suggests that drug release from these formulations presents a poor process. In addition, the rest of the formulations showed r values greater than the first order, indicating that drug release follows the first order and drug release from microcapsules is both diffusion and erosion.

.

Table 4: Drug release kinetics of clopidogrel microcapsules

Formulation	R^2				Peppas N
	Zero	First	Higuchi	Peppas	
F1	0.8998	0.9200	0.9975	0.9971	0.481
F2	0.8664	0.9681	0.9811	0.9525	0.455
F3	0.7819	0.986	0.9607	0.9833	0.355
F4	0.9186	0.9867	0.9873	0.9557	0.628
F5	0.8592	0.9155	0.9526	0.9380	0.602
F6	0.8374	0.9824	0.9790	0.9800	0.428
F7	0.9744	0.9316	0.8884	0.9843	1.072
F8	0.9884	0.9818	0.9260	0.9858	1.073
F9	0.9763	0.9561	0.9439	0.9916	0.922

Experimental design validation

The release of clopidogrel bisulfate at the 1-hour and 8-hour time points was considered a measurable parameter for the DOE experiment. The 1-hour period represents the oscillation rate and the 8-hour period measures the oscillation rate. There is a positive correlation between the ratio of drug to EC and the amount and amount of drug released over 1 and 8 hours. HPMC 5 cps added because the pore had previously been found to be neither efficient nor effective for drug release. However, for the sample containing higher amount of HPMC, the drug release was more complete (at higher EC level) compared to the sample with less HPMC level. DOE plans are obtained by entering encapsulation efficiency data into DOE.Pro XL software and the remainder is used to calculate the cost of building the space. The independent variables for preparation of the Y-hat contour plot, Y-hat surface area, and Y-hat interaction plot are clopidogrel bisulfate, ethylcellulose, and HPMC. The graph shows a positive interaction between clopidogrel bisulfate and EC and HPMC.

Figure 5: Contour plot of %DR at 1hr

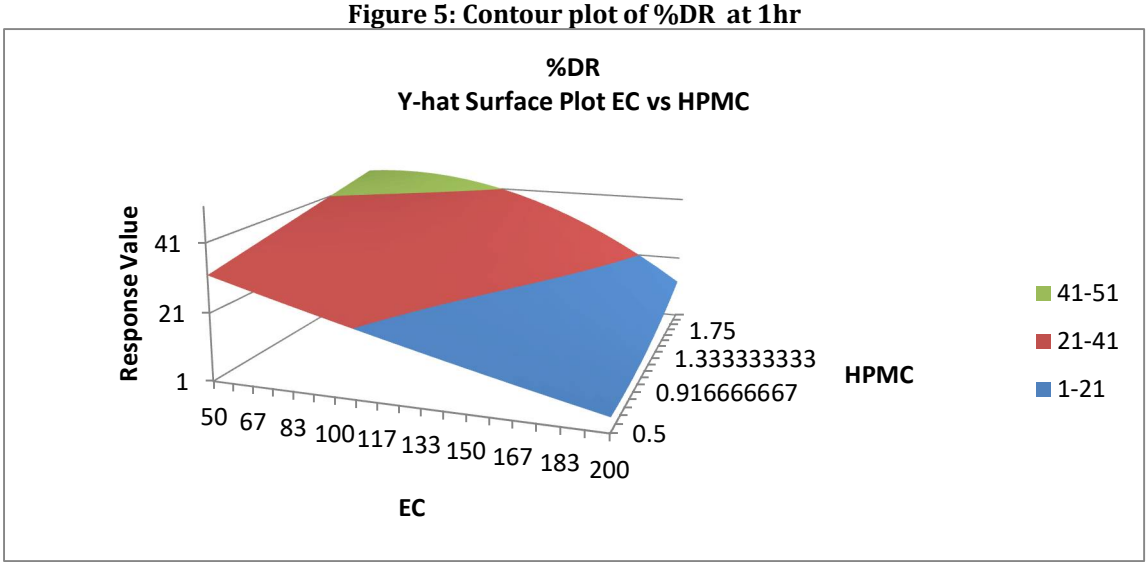
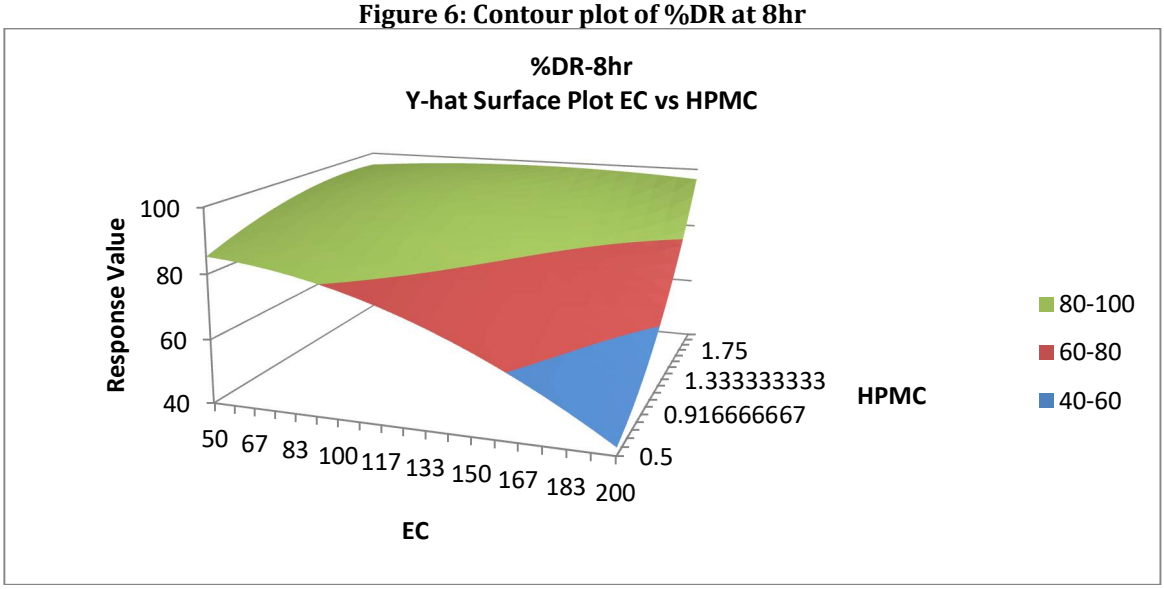


Figure 6: Contour plot of %DR at 8hr



CONCLUSION

Entrapment efficiency is one of the most important characteristic of particulate drug delivery systems and it decides the weight of the formulation to be taken in order to have required dose. In this work, influence of various formulation and process parameters on entrapment efficiency and particle was aimed to explore so as to develop microcapsules with good entrapment efficiency. The obtained results were analyzed by ANOVA and found that all the selected factors were found to have significant influence on entrapment efficiency and hence the major objective of the work was achieved.

CONFLICT OF INTEREST

No conflicts of interests are disclosed by the authors.

REFERENCES

1. Praveen Kumar, G., Anand, J. (2011). Porous floating microspheres: A new dimension in controlled drug delivery. Research J. Pharm. and Tech., 4(9): 1340 – 1357.
2. Shivalingam, MR., Anil Kumara, KSG., Kishore Reddy, YV. (2010). Formulation and evaluation of floating microballoons of ibuprofen for the enhanced enteric bioavailability. Research J. Pharm. and Tech., 3(2): 583 – 585.
3. Patil, UK., Ravish Sahu., Yadav, SK. (2009). Formulation and Evaluation of Controlled Release Microspheres Containing Metformin Hydrochloride. Research J. Pharm. and Tech., 2(1): 176 – 179.
4. Mohanraj, P., Jasmina Khanam., Arun Kumar, N., Rani,C. (2009).Chitosan Microspheres Encapsulated With Metoprolol Succinate: Formulation and In - Vitro Evaluation. Research J. Pharm. and Tech., 2(2): 349 – 352.
5. Swathi, V., Velrajan, G., Balasubramaniam. (2013). Formulation and Evaluation of Albumin Microspheres containing Stavudine., 6(2): 174 – 177.

6. Gangadhar, B., Shyam Sunder, R., Vimal Kumar Varma, M., Sleeva Raju, M., Sai Kiran, M. (2010). Formulation and Evaluation of Indomethacin Microspheres using Natural and Synthetic Polymers as Controlled Release Dosage Forms. International Journal of Drug Discovery., 2(1): 08-16.
7. Zhenqiu Yang., Baozhen Song., Qiaoxia Li., Honglei Fan., Fan Ouyang. (2004). Preparation of Microspheres with Microballoons Inside for Floating Drug-Delivery Systems, Journal of Applied Polymer Science., 94, 197–202.
8. Bhaskar Mazumder., Mrinal Kanti Sarkar., Sanjay Dey., Nibedita Roy. (2010). Effect of Formulation and Process Variables on the Characteristics of Microspheres of Anti-Viral Drug (stavudine) Prepared by Oil-in-Oil Solvent Evaporation Technique. International Journal of Pharmacy and Pharmaceutical Sciences., 2(2).
9. Kumaresh, S., Anandrao, R., Walter, E., Tejraj, M. (2001). Microspheres as floating drug delivery systems to increase gastric retention of drugs, DMR., 33(2), 149–60.
10. Prashant, M., Upendra, N., Raj Kaur, M., Neha, G. (2013). Pentoxifylline Loaded Floating Microballoons: Design, Development and Characterization, Journal of Pharmaceutics., 1(5).
11. Kumar, S., Nagpal, K., Singh, SK., Mishra, DN. (2011). Improved bioavailability through floating microspheres of lamivudine. DJPS., 19, 57-64.
12. YogeshMukund, J., RohanKantilal, B., Nisharani Sudhakar, R. (2012). Floating microspheres: A Review. Brazilian Journal of Pharmaceutical Sciences., 48, 17-30.
13. Rishikesh Gupta., Sunil Kumar, P., Snigdha Pattnaik., Peeyush Bhardwaj. (2014). Formulation and evaluation of novel stomach specific floating microspheres bearing famotidine for treatment of gastric ulcer and their radiographic study. Asian Pac J Trop Biomed., 4(9): 729- 735.
14. [14]. Praveen Kumar, G., Shikha, M., Meenakshi, B. (2014). Formulation and evaluation of controlled-release of telmisartan microspheres: In vitro/in vivo study. Journal of Food and Drug Analysis., 22, 542- 548.
15. Jain, D., Panda, AK., Majumdar, DK. (2005). Eudragit s100 entrapped Insulin Microspheres for Oral Delivery. AAPS Pharm. Sci. Tech., 6:e100-7.
16. Jain, SK., Agrawal, GP., Jain, NK. (2006). Evaluation of porous carrier-based floating orlistat microspheres for gastric delivery. AAPS Pharm. Sci. Tech., 7:E54-62.
17. Dupinder, K., Seema, S. (2014). Development and Characterization of Lovastatin Microspheres. IJPDT., 4(2): 71-8.
18. Mukund, JY., Kantilal, BR., Sudhakar, RN. (2012). Floating microspheres: a review. Br JPharm Sci., 48(1):17-30.
19. Gidwani, B., Vyas, A., Ahirwar, K., Shukla, S. (2016). Factorial design and a practical approach for gastro – retentive drug delivery system. Research J. Pharm. and Tech., 9(4): 641-9.

CITATION OF THIS ARTICLE

S. Ramya Krishna, B. Sowjanya Lakshmi, Y. Sailaja, A. Ramu, S. Vidyadhara. Optimization, Development and Characterization of Clopidogrel Bisulphate Microcapsules by DoE . Bull. Env.Pharmacol. Life Sci., Vol 13 [4] March 2024: 17-23