Journal of Research in Pharmacy

Research Article

www.jrespharm.com

Optimization of Parameters for The Design and Evaluation of Fenofibrate Loaded Floating Microspheres by Taguchi Method

Josephine LENO JENITA [1] *, Mahesh AGASA RAMU [2], Priyanka SAH [1], Seema S RATHORE[1]

[1] College of Pharmaceutical Sciences, Dayananda Sagar University, Pharmaceutics, Bengaluru, Karnataka, India.
[2] Faculty of Pharmacy, MS Ramaiah University of Applied Sciences, Pharmaceutical Chemistry, Bengaluru, Karnataka, India.
* Corresponding Author. E-mail: jenita79@gmail.com (J.L.J); Tel. +91-944-842 92 14.

Received: 19 July 2022 / Revised: 16 September 2022 / Accepted: 16 September 2022

ABSTRACT: Fenofibrate is an anti-hyperlipidemic agent with poor water solubility and poor bioavailability of 30%. The goal of this study was to develop and optimise floating microspheres of Fenofibrate utilising an emulsion solvent diffusion method in order to improve absorption and oral bioavailability of the medicine for a better approach in treating hyperlipidemic conditions using ethyl cellulose as a polymer. At the preliminary stage four formulations were prepared by changing the polymer ratio and keeping the stirring speed, stirring time and solvent composition constant. Based on the results obtained batch 2 was considered as an ideal batch. The data from this batch was used at the middle level of a Taguchi orthogonal array design to optimise the formulation, and the effect of independent variables such as A (Polymer concentration), B (Stirring speed), C (Stirring time), and D (Ethanol concentration) on dependent variables such as particle size, percentage yield, drug loading, buoyancy, and drug release was investigated. All the microspheres showed good buoyancy for 24 h in simulated gastric fluid and controlled release of drug for 12 hours. The optimized formulation was spherical in shape as confirmed by photographs from scanning electron microscopy. The *in vitro* release data were fitted into various kinetic models and the possible release mechanism was found to follow Korsmeyer-Peppas model. The results suggest that Fenofibrate floating microspheres provides modified drug release for treating hyperlipidemia and can be used successfully for development of sustain release formulation.

KEYWORDS: Fenofibrate; Ethyl Cellulose; Eudragit RL100; Anti-hyperlipidemic; Taguchi design.

1. INTRODUCTION

The primary goal of any drug regimen is to achieve a stable plasma drug concentration or tissue concentration that is nontoxic and pharmaceutically efficacious over time. The oral route of drug delivery has received the most attention among the various routes of drug administration, owing to its ease of administration and capacity to be flexible with dosage form. Modified drug release systems, such as controlled drug release systems, site-specific drug release systems, delayed drug release systems, and sustained drug release systems, overcome many of the shortcomings of traditional oral dose forms [1, 2]. The use of gastro-retentive dosage forms is one of the most feasible strategies for achieving a sustained and predictable medication distribution in the gastrointestinal system [3]. Gastro retentive drug delivery systems (GRDDS) stay in the stomach for a longer period of time, improving the medication's gastric residence duration and boosting bioavailability and solubility of less soluble pharmaceuticals while reducing drug waste [4]. A floating drug delivery system (FDDS) is a GRDDS in which low-density systems have enough buoyancy to float above the gastric juice and remain in the stomach for extended period of time, resulting in increased GRT and reduced plasma drug variations [5, 6]. Floating microspheres are FDDS and are free-flowing particles with a diameter of less than 200 micrometers that float for an extended period of time in the stomach fluid [7].

Fenofibrate, an anti-hyperlipidemic medication, is a fibrate class derivative of third generation fibric acid. Fenofibrate is classified as a Class II medicine by the Biopharmaceutics classification system (BCS), due to its poor solubility and high permeability which results in low bioavailability. It aids in the reduction of high triglyceride and low-density lipoprotein levels [8]. It acts by stimulating the activity of peroxisome-activated-

How to cite this article: Leno Jenita J, Agasa Ramu M, Sah P, Rathore SS. Optimization of parameters for the design and evaluation of fenofibrate loaded floating microspheres by Taguchi method. J Res Pharm. 2023; 27(1): 130-144.

ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.298

alpha receptor, which is a member of the Peroxisome proliferator-activated receptor subfamily of nuclear receptors that modulate the transcription of genes that regulates fatty acids and cholesterol metabolism. The drug's dose ranges from 140 to 200 mg twice a day, with a biological half-life of 6 to 8 h and a low oral bioavailability of 30%, necessitating frequent dosing to maintain the drug's therapeutic impact, therefore in the current study Fenofibrate was chosen as a model drug to maintain a sustained drug concentration in the body for prolonged period of time, which will result in enhanced absorption thereby, improving the bioavailability.

2. RESULTS AND DISCUSSION:

2.1. Experimental design

Taguchi's optimization technique is a factorial design used to optimize the floating microspheres. This model is distinctive and powerful, it reduces the cost, improves the quality and provides robust design and also produces minimum number of experiments. Many factors can be modified simultaneously utilizing Taguchi's methodology, and quantitative information can be collected from fewer experimental trials [9].

2.2. Preparation of microparticles

Nine different batches of fenofibrate microparticles were prepared by emulsion-solvent diffusion method. This method was chosen since it is easy, quick, and cost-effective. Heat, high-energy, and high-cost apparatus are not used in this method. Ethyl cellulose was preferred as a polymer as it was reported to have sustained release property [10]. Polyvinyl alcohol was used in the preparation as a stabilizer, it gave spherical shaped microspheres without agglomeration [11]. Ethanol and dichloromethane (DCM) are used as solvents because of their volatile nature, and they also prevent polymer precipitation [11, 12]. Ethanol itself is a good solvent and non-toxic in nature and it is reported to produce perfectly spherical shaped particle with a smooth surface [13].

2.3. Characterization of microspheres

2.3.1. Compatibility studies

FTIR Spectra

The FTIR spectra of Fenofibrate, ethyl cellulose, physical mixture of Fenofibrate and ethyl cellulose and Fenofibrate loaded Ethyl Cellulose microspheres (**Figure** 1) were recorded and compared (**Table 1**).

Table 1. IR interpretation of FT-IR spectrum of drug, physical mixture and formulation of drug polymer

Name of the compound	-OH	-CH	-C=O	-C=O	-NH-	-CO
Fenofibrate	2983.14	2881	1724.71	1648.68	1596.14	1273.57
Fenofibrate + Ethyl Cellulose (Physical mixture)	2980.74	2884.76	1727.44	1650.17	1597.86	1285
Fenofibrate loaded Ethyl Cellulose microspheres	2980.13	2878	1725.45	1648.85	1596.11	1284.56

2.3.2. Percentage yield:

Percentage yield Table **3**) was increased with respect to increase in polymer concentration and stirring time as seen in one factor response graph and with increase in ethanol concentration it reduced (**Figure 2**) and it was explained by polynomial equation.

$$\% \text{ yield} = +77.97+5.60*A\,[1] +1.08*A\,[2]-2.65*C\,[1] +0.6022*C[2]+2.42*D[1]-2.35*D[2]$$

The response was analyzed by the Factorial model which showed F value of 30.24 and p value of 0.0324 (Table 2) indicating a significant response. Factor A- Polymer concentration had positive effect on the % yield, factor C- Stirring time showed negative influence at lower limit and positive at higher limit and factor D- Ethanol concentration had a positive influence with low concentration and negative effect at high concentration. The

predicted and actual values of percentage yield were correlated and were close to each other. The response was further elucidated with the 3D response surface plots (**Figure 3**).

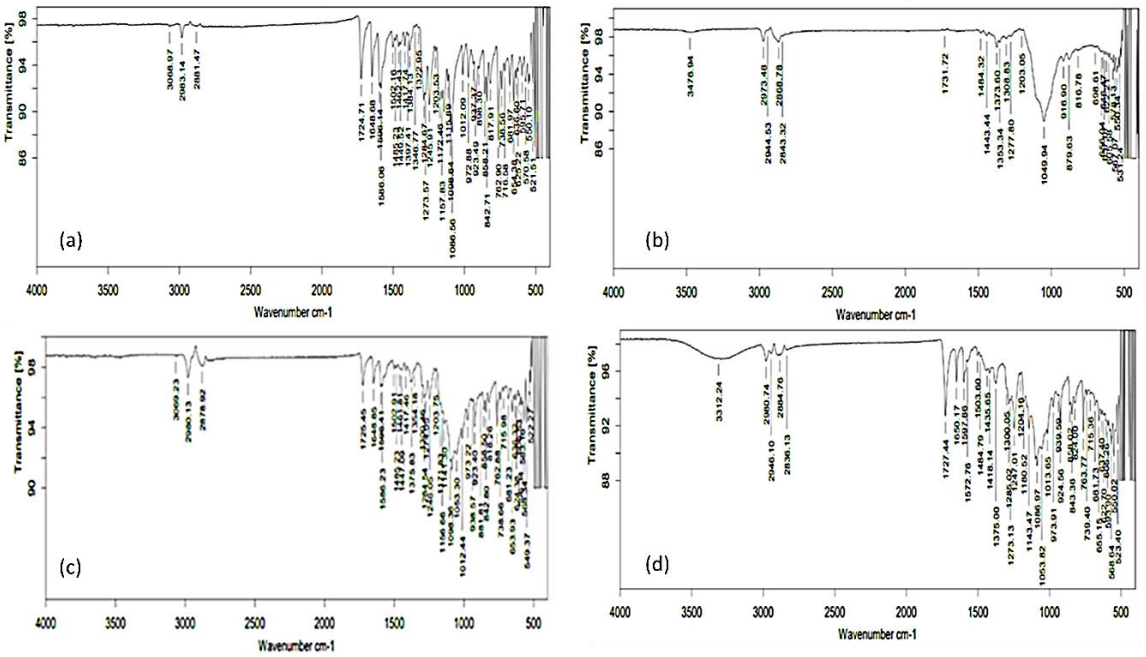

Figure 1. IR spectrum of (a) pure drug (Fenofibrate), (b) Ethyl cellulose, (c) Physical mixture of drug + Ethyl cellulose, (d) Fenofibrate loaded Ethyl Cellulose microspheres

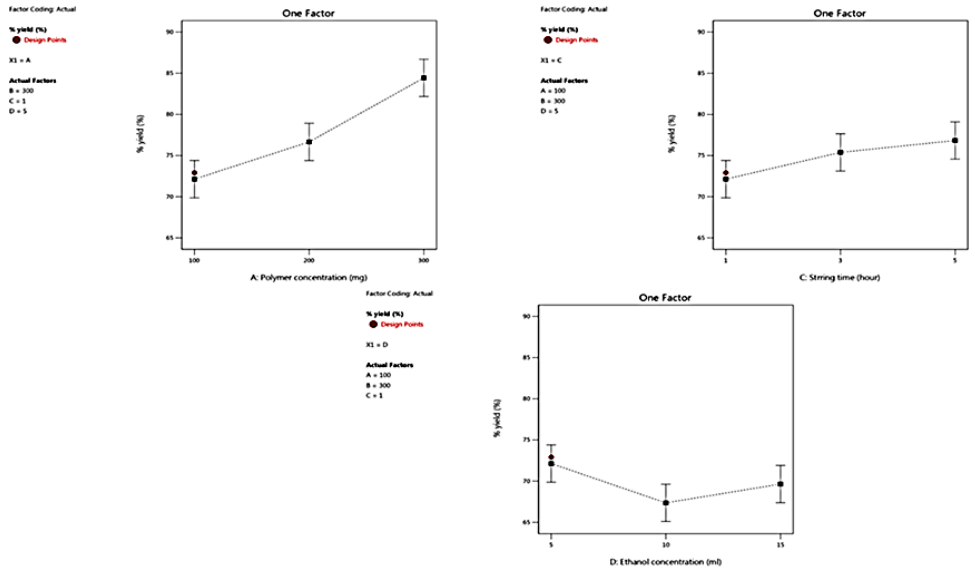

Figure 2. One factor response graph of (a) Polymer concentration, (b) stirring time and (c) ethanol concentration against Percentage yield

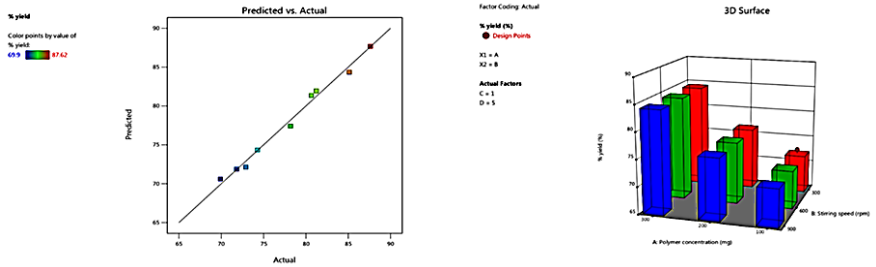

Figure 3. Predicted v/s actual correlation and 3D response graph of Particle size

The increase in the percentage yield may be due to the increased amount of the polymer slurry and further decrease may be due to the agglomeration and sticking of polymers to beads of the stirrer and wall of the beaker with rapid evaporation of solvent during formation of microspheres [14]. With increase in ethanol concentration process yield decreased, it is due to the fast diffusion of the ethanol in the water before formation of the droplets [15].

2.3.3. Drug loading

Drug loading (**Figure 4**) was adversely affected by Polymer concentration and stirring speed. As the factor A and B enhanced, the drug loading reduced (**Figure 4**) and was explained by polynomial equation.

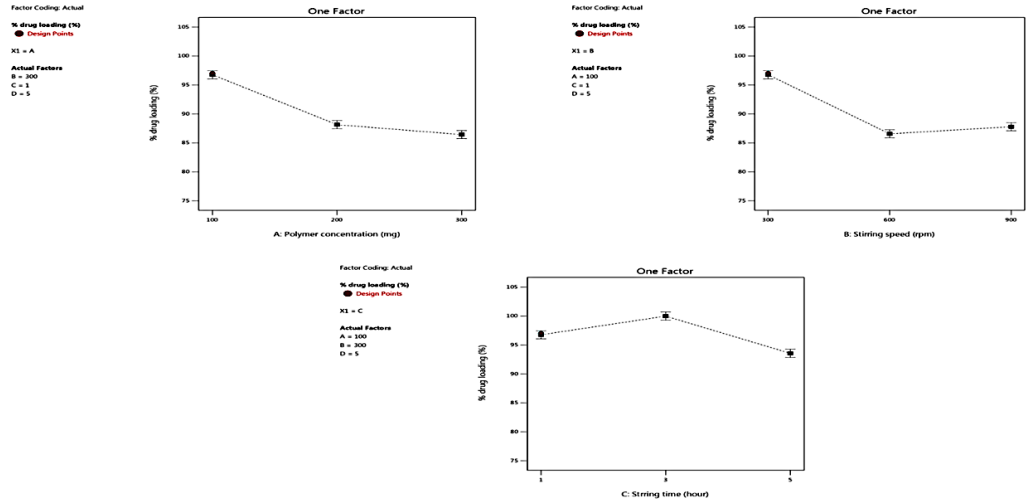

Figure 4. One factor response graph of (a) Polymer concentration, (b) stirring time and (c) ethanol concentration against % drug loading

$$\% \text{ Drug loading} = +84.05 + 6.32*A\,[1] - 2.30*A\,[2] + 6.39*B\,[1] - 3.81*B\,[2] - 0.0044*C\,[1] + 3.21*C\,[2]$$

The response was analyzed by a Factorial model which showed F value of 452.27 and p value of 0.0022 (Table 2) indicating a significant response. Factor A at low levels showed highest drug loading, whereas at higher level the drug loading efficiency was reduced. Factor B had negative effect at high level and positive effect at low level and factor C at intermediate time, increased the drug loading efficiency when compared to lower and higher levels. The predicted and actual values of drug loading were correlated with each other and were in good co-ordination with each other. The response was further interpreted with the 3D response surface plots (**Figure 5**).

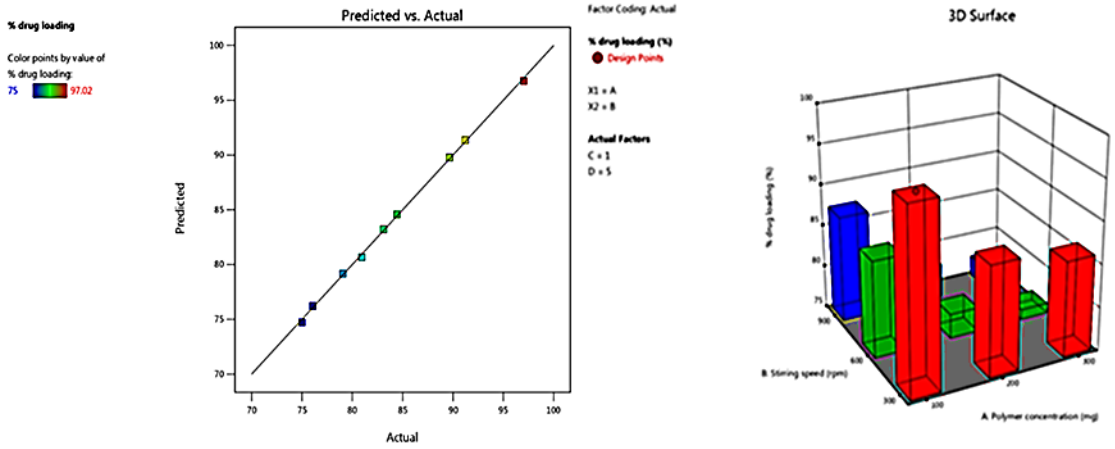

Figure 5. Predicted v/s actual correlation and 3D response graph of % drug loading

Increased polymer concentration in the internal phase showed reduction in drug loading. This may be due to decrease in viscosity of internal phase which enhances the migration of drug in aqueous phase. Thus, viscous polymer used during formulation shows less drug loading [16]. Drug loading decreasing with increasing

stirring rate from 300 to 900rpm, may be due to smaller size microspheres formed at higher speed of rotation. Also, loss of drug from surface of small particles is more as compared to larger one during washing of microspheres [17].

2.3.4. Particle size

Particle size (Table **3**) was increased with increase in polymer concentration and decreased with increase on stirring speed as shown in one factor response graph (**Figure 6**) and it was explained by polynomial equation.

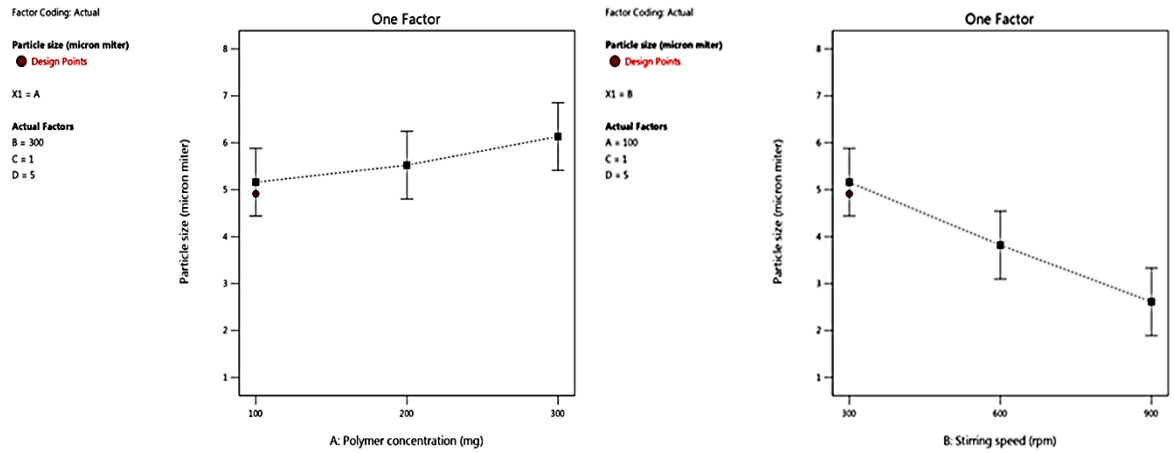

Figure 6. One factor response graph of (a) polymer concentration and (b) stirring speed against particle size.

$$\text{Particle size} = +4.31 - 0.4451*A\ [1] - 0.0828*[2] + 1.30*B\ [1] - 0.0444*B\ [2]$$

The response was analyzed by the Factorial model which showed F-value of 6.92 and p-value of 0.0438 (Table 2) indicating a significant response. Factor A- as the polymer concentration increased the particle size of microsphere also increased and Factor B-Stirring speed had a negative effect on the particle size i.e., as the stirring speed increased, the particle size decreased. The actual and predicted values of particle size were correlated as shown in the **Figure 7** The particle size of the microspheres was found to be in the range of 2.504 to 6.255 µm and were suitable for oral administration. The response was further elucidated with the response surface plots **Figure 7**.

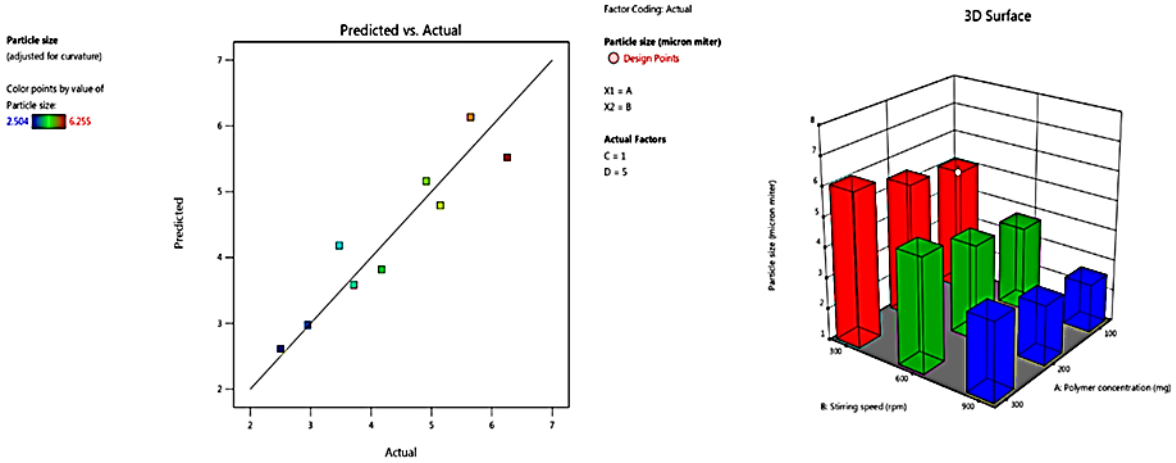

Figure 7. Predicted v/s actual correlation and 3D response graph of particle size

The size of the microspheres increased with increase in polymer concentration, this may be due to increase in the viscosity of feed solution which results in the formation of larger size polymer droplets and thus results in larger size particles [13]. The mean particle size of the microspheres was found to be reduced significantly with increase in stirring rate because at high speed (i.e., greater mechanical stress) small droplets of emulsion are formed which results in breakdown of particles [18].

2.3.5. % Buoyancy

Floating ability (Table 3) of the microspheres was found to increase with increase in polymer concentration and at middle level of stirring speed and time the % buoyancy decreased, and further increase led to increase (**Figure 8**) and was demonstrated with the polynomial equation.

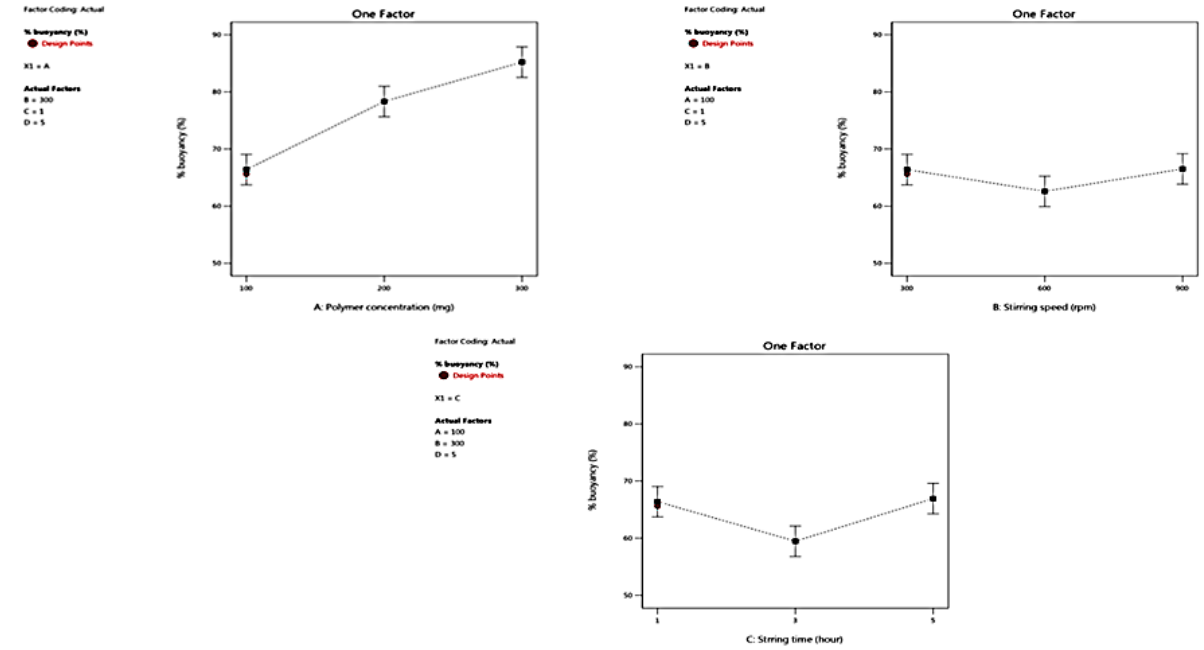

Figure 8. One factor response graph of (a) polymer concentration, (b) stirring speed and (c) stirring time against % buoyancy

$$\% \text{ buoyancy} = +73.29 - 10.25*A[1] + 1.68*A[2] + 1.23*B[1] - 2.58*B[2] + 2.13*C[1] - 4.80*C[2]$$

The response was analyzed by Factorial model which showed F value of 49.16 and p value of 0.0201 (**Error! Reference source not found.**) indicating a significant response. The factor A at high levels showed the positive influence on floating ability whereas Factor B and C showed positive influence at lower and high level with decrease in buoyancy at middle level. The predicted and actual values of floating ability were correlated and were in good relation with each other (**Figure 9**). The response was further elucidated with the response surface plots (Error! Reference source not found.**9**).

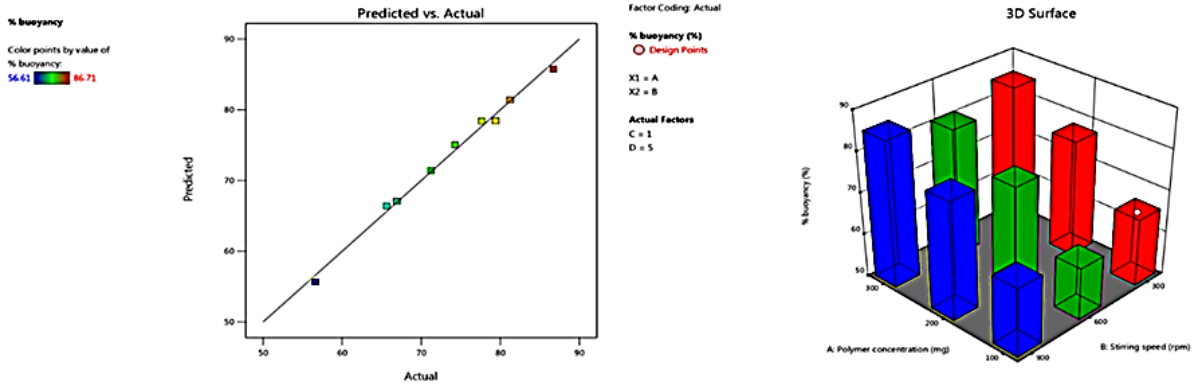

Figure 9. Predicted v/s actual correlation and Response graph of % buoyancy

The continuous increase in % buoyancy with an increase in ethyl cellulose proportion could be due to the hydrophobic nature of the polymer (insolubility of the ethyl cellulose polymer in 0.1N HCl) which decrease the penetration of the medium into the microspheres. Reason for less buoyancy with increase in stirring speed and time might be due to increased amount of absorbed liquid medium replacing the air inside the microspheres rendering them less buoyant [19].

2.3.6. Drug release

Drug release at 12 h was found to increase with increase in stirring time, but further increase, led to reduction in effect. The drug release dropped when both ethanol and polymer concentration increased (**Error! Reference source not found.**) and it was explained by the polynomial equation.

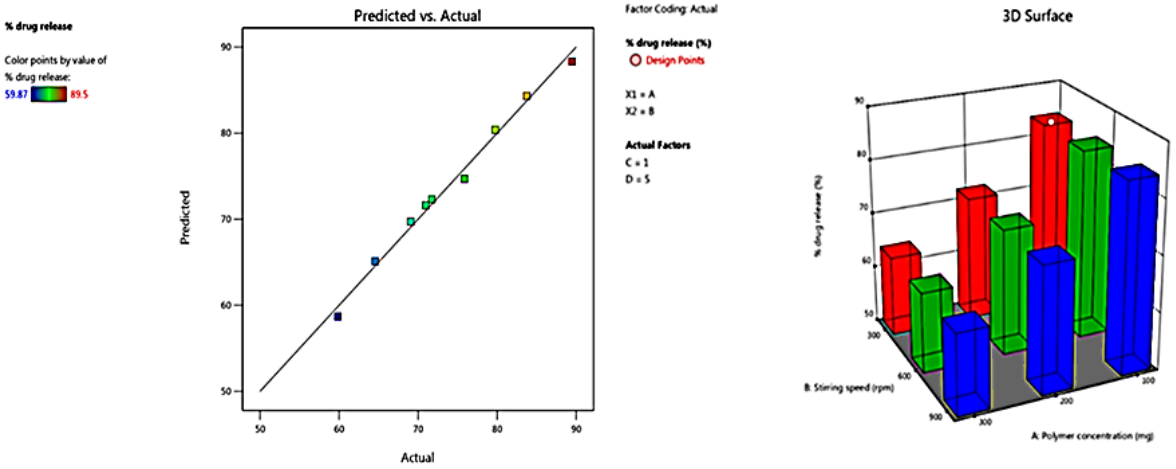

Figure 10. One factor response graph of (a) Polymer concentration, (b) stirring time and (c) ethanol concentration against drug release

$$\% \text{ drug release } = +73.90 + 10.44*A\,[1] - 1.04*A\,[2] - 2.37*C\,[1] + 2.87*C[2] + 2.33*D[1] + 1.11*D[2]$$

The factorial model was used to analyze the response and the model was found to be significant with F value of 37.70 and p value of 0.0265 (Table 2). Factor A-Polymer concentration was seemed to have positive effect at the low level and negative effect at the high level. From this it was evident that increase in polymer concentration showed decrease in % drug release. Lower stirring time (Factor-C) showed negative influence on response whereas middle concentration had shown increased response as compared to higher concentration. Factor D-Ethanol had a positive influence on the response. The predicted and actual values of % drug release were correlated and were close enough with each other. The response was further elucidated with the 3D response surface graph (**Error! Reference source not found.**).

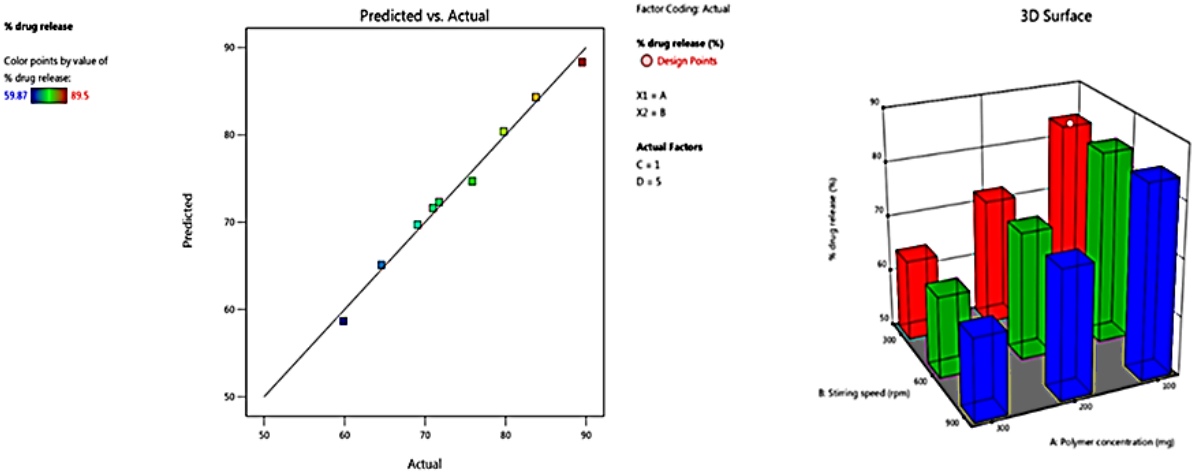

Figure 11. Predicted v/s actual correlation and 3-D Response surface of % drug release

The percent drug release was found to be decreased with increase in polymer concentration because the drug release from the microspheres was controlled by the polymer. Ethyl cellulose is water insoluble polymer, they prevent the penetration of dissolution medium into the microspheres leading to slower dissolution and diffusion of drug molecules. With increase in stirring time, particle size decreases and leading to higher surface area exposure for the dissolution medium. This results in enhanced dissolution and gives more drug release (**Table 2 and Figure 12**) [20].

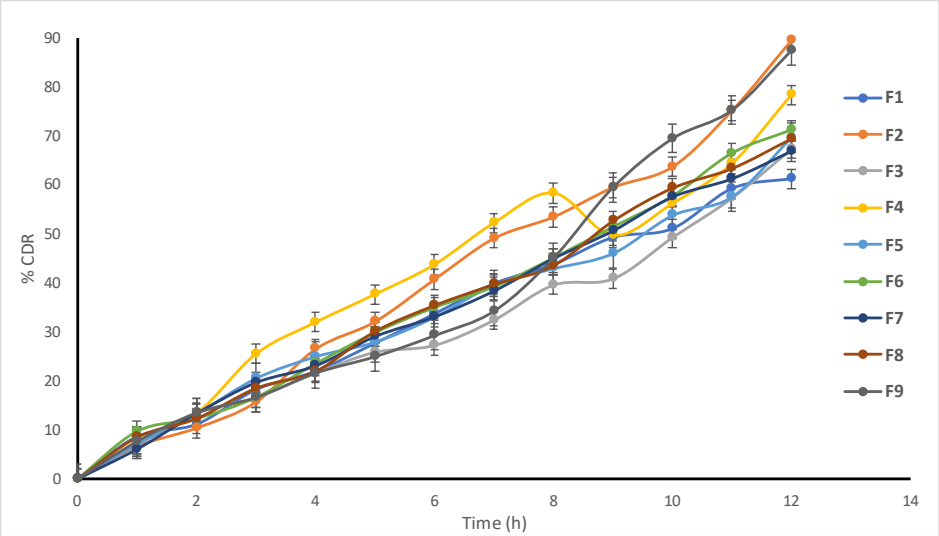

Figure 12. Cumulative percentage drug release of F1 to F9

Table 2. Summary of ANOVA results of selected factorial model of Fenofibrate loaded floating microspheres with Ethyl cellulose for particle size, percentage yield, drug loading, buoyancy and drug release.

Responses	Source	Sum of Squares	df	Mean Square	F-value	p-value
Particle size	Model	11.20	4	2.80	6.92	0.0438*
	A-Polymer concentration	1.45	2	0.7255	1.79	0.2780
	B-Stirring speed	9.75	2	4.87	12.05	0.0203
	Residual	1.62	4	0.4046		
	Cor Total	12.82	8			
Percentage yield	Model	300.90	6	50.15	30.24	0.0324*
	A-Polymer concentration	231.98	2	115.99	69.93	0.0141
	C-Stirring time	34.67	2	17.34	10.45	0.0873
	D-Ethanol concentration	34.25	2	17.12	10.32	0.0883
	Residual	3.32	2	1.66		
	Cor Total	304.22	8			
Drug loading	Model	431.68	6	71.95	452.27	0.0022*
	A-Polymer concentration	183.89	2	91.95	577.99	0.0017
	B-Stirring speed	185.96	2	92.98	584.50	0.0017
	C-Stirring time	61.82	2	30.91	194.32	0.0051
	Residual	0.3182	2	0.1591		
	Cor Total	432.00	8			
% Buoyancy	Model	678.02	6	113.00	49.16	0.0201*
	A-Polymer concentration	543.99	2	271.99	118.33	0.0084
	B-Stirring speed	30.05	2	15.03	6.54	0.1327
	C-Stirring time	103.98	2	51.99	22.62	0.0423
	Residual	4.60	2	2.30		
	Cor Total	682.62	8			
Drug release	Model	692.84	6	115.47	37.07	0.0265*
	A-Polymer concentration	594.89	2	297.44	95.49	0.0104
	C-Stirring time	42.33	2	21.17	6.79	0.1283
	D-Ethanol concentration	55.62	2	27.81	8.93	0.1007
	Residual	6.23	2	3.11		
	Cor Total	699.07	8			

*** Significant**

Table 3. Optimization Results of Particle size, Percentage yield, Drug loading and Buoyancy for Fenofibrate loaded Ethyl cellulose microspheres

Formulation	Percentage yield (%)	Drug loading (%)	Particle size (µm)	Buoyancy (%)
F1	72.91	75	3.716	65.62
F2	69.9	87.01	4.175	56.61
F3	78.19	84.44	5.648	66.9
F4	74.28	91.21	6.255	46.29
F5	89.64	97.02	3.472	86.34
F6	82.15	79.05	2.952	59.4
F7	85.12	83.1	5.147	74.28
F8	79.04	76.08	2.504	54.61
F9	79.81	80.94	4.913	77.64

Table 4. *In vitro* release data of Fenofibrate loaded Ethyl cellulose microspheres (F1-F9)

Sl.no	Time (hour)	% Cumulative drug release								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
1	0	0	0	0	0	0	0	0	0	0
2	1	8.64	6.64	7.18	7.16	7.04	9.72	6.08	8.51	7.66
3	2	11.16	11.39	13.48	13.37	13.34	12.33	13.37	12.33	13.48
4	3	18.24	15.72	16.64	25.54	20.54	16.65	19.67	18.52	16.64
5	4	21.97	26.51	21.62	31.98	24.98	23.52	23.10	21.99	21.62
6	5	27.70	32.12	25.89	37.70	27.97	29.87	29.08	30.12	24.99
7	6	33.71	40.76	27.30	43.78	33.05	34.99	33.10	35.44	29.30
8	7	39.87	49.14	32.47	52.29	39.67	39.21	38.38	39.73	34.30
9	8	43.78	53.52	39.63	58.37	42.98	45.12	45	43.59	45.16
10	9	49.29	59.54	40.90	49.70	46.09	51.44	50.67	52.69	59.59
11	10	51.10	63.76	49.34	56.24	53.87	57.73	57.58	59.42	69.58
12	11	59.29	75.25	57.42	64.37	57.65	66.50	61.31	63.42	75.38
13	12	61.32	89.65	67.47	78.45	69.8	71.27	66.93	69.51	87.61

2.4. Regression analysis

Numerical optimization was used to optimize the formulations. For both dependent and independent variables, optimal settings were determined, and the response variables were evaluated by factorial model. The model p values and F values for particle size was 0.043 and 6.92, for % yield 30.24 and 0.0324, for drug loading 452.27 and 0.0022, for % buoyancy 49.16 and 0.0201, drug release for 37.07 and 0.0265 (Table 2) The models were found to be significant as the p values were less than 0.0500. The R^2 values of particle size, % yield, drug loading, % buoyancy and drug release were 0.912, 0.893, 0.973, 0.958 and 0.962 respectively. The greater the regression values, more contribution was observed on significance of factors. With the help of predicted model, the optimized formulations were prepared and evaluated for the responses. The predicted and actual values were analysed, and it was found that, both were in close relation (**Error! Reference source not found.**).

Table 5. Actual and predicted values of optimized formulation

Formulation	Ethyl Cellulose microspheres (F2)	
Variables	**Predicted**	**Actual**
Particle size	3.81956	4.175
Percentage yield	70.6111	69.9
Drug loading	87.7711	87.01
Buoyancy	55.6633	56.61
Drug release	88.3244	89.5

2.5. Evaluation of optimized formulation

2.5.1 Compatibility studies, FTIR Spectra

The main peaks of pure Fenofibrate were seen in the microsphere's spectra along with other peaks which were prominent in ethyl cellulose. The obtained peaks indicated that the medication and polymer were compatible (**Figure 13**)

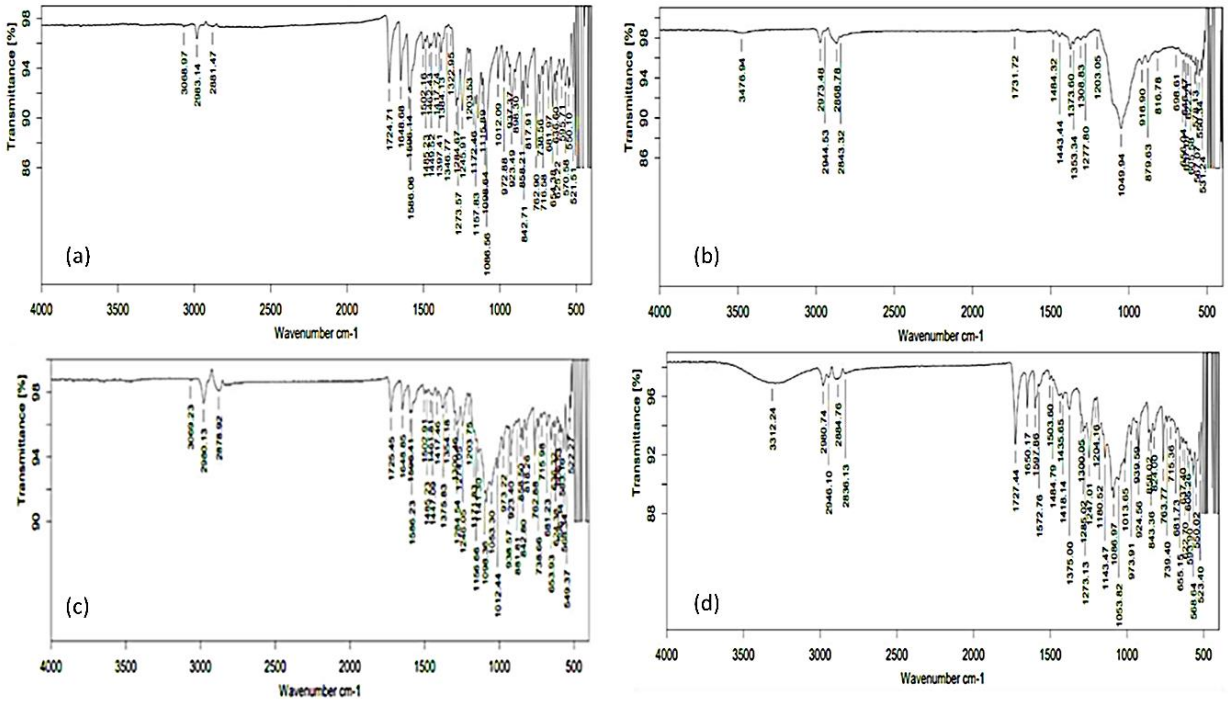

Figure 13. IR spectrum of (a) pure drug (Fenofibrate), (b) Ethyl cellulose, (c) Physical mixture of drug + Ethyl cellulose, (d) Fenofibrate loaded Ethyl Cellulose microspheres

2.5.2. Scanning Electron Microscopy (SEM)

The surface morphology of Fenofibrate microspheres was observed under scanning electron microscopy. Microspheres had good spherical structure with a smooth surface morphology as confirmed by photographs (**Figure 14**)

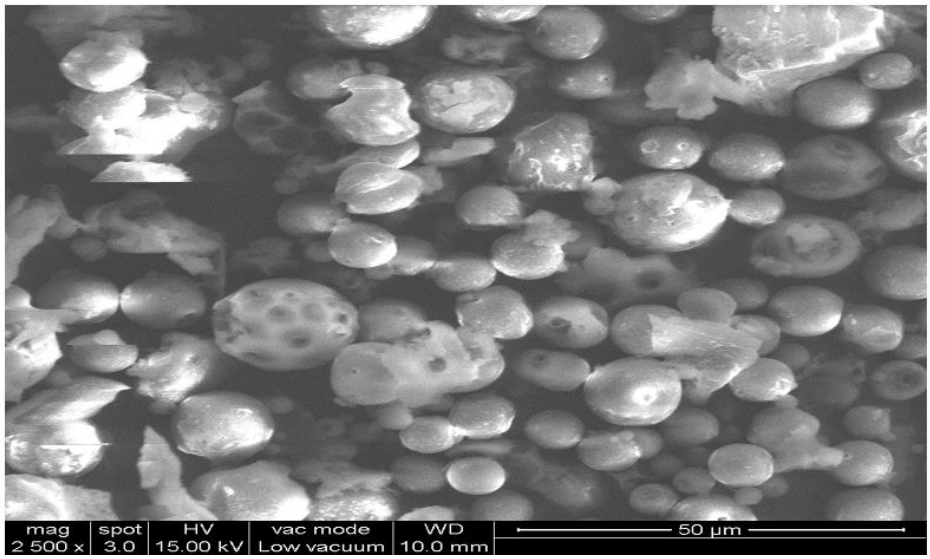

Figure 14. Scanning electron micrograph of Fenofibrate loaded Ethyl cellulose Floating microspheres (F2)

2.5.3. *In vitro* drug release kinetics

The optimized formulations obtained from *in vitro* release data was fitted to different kinetic models such as zero order, first order, Higuchi model and Korsmeyer-Peppas model (**Figure 15 and Table 6**). The kinetic model fitting data showed that the release of drug from microspheres followed Korsmeyer-Peppas model and the formulations followed the super case-II transport which indicated that drug release from floating microspheres was by diffusion controlled polymeric relaxation.

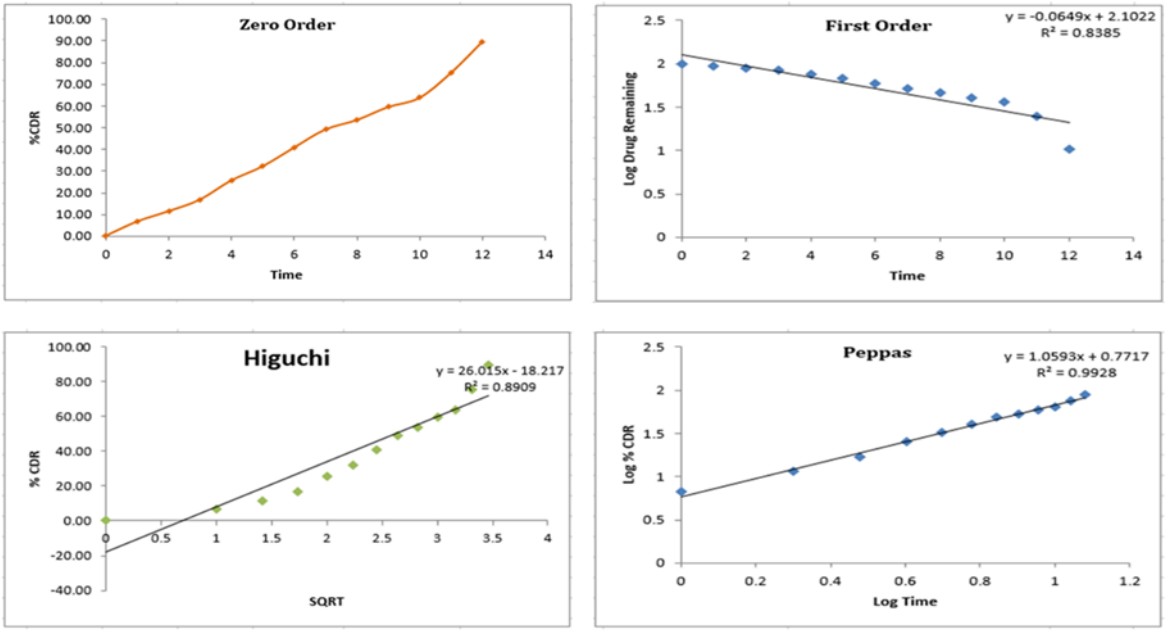

Figure 15. Release kinetic graph of optimized formulation of Fenofibrate loaded Ethyl cellulose microspheres (F2)

Table 6. Release kinetic data of optimized formulation of Fenofibrate loaded Ethyl cellulose microspheres (F2)

Formulation	Zero order	First order	Higuchi matrix	Korsmeyer-Peppas model	
				r^2	N
F2	0.9898	0.8385	0.8908	0.9928	1.059

3. Conclusion

The current research study was a satisfactory attempt in formulating floating microspheres of an anti-hyperlipidaemic drug such as Fenofibrate to enhance its bioavailability. From FTIR spectra study, the drug and polymer were found compatible with each other. The preliminary study of particle size, percentage yield, drug loading, % buoyancy and drug release indicated that A2 was the ideal batch. By taking A2 as the medium variable in Taguchi model, the optimized formulation was determined. According to Taguchi design results, particle size, percentage yield, drug loading, percentage yield, and drug release were all significantly affected by polymer concentration, stirring speed, stirring time, and ethanol content. At the end of 12 hours, the optimised formulation (F2) had a drug release of 89.65 percent, and the formulation had been floating for more than 24 hours. Scanning electron microscopy photographs confirmed that the microspheres had an excellent spherical structure with a smooth surface morphology. The best fitted model was Korsmeyer-Peppas model demonstrating that drug release followed the super case-II transport and that drug release from floating microspheres was controlled by diffusion controlled polymeric relaxation.

4. MATERIALS AND METHODS

4.1. Materials

The drug Fenofibrate was procured from Yarrow Chem Products, Mumbai, India. Ethyl Cellulose and ethanol was obtained from SD Fine Chemicals Ltd. Mumbai, India. Di Chloro methane and Poly vinyl alcohol was procured form Sigma Aldrich, Bangalore. All the chemicals and reagents used were of analytical quality.

4.2. Preparation of floating microspheres

The floating microspheres loaded with Fenofibrate was prepared by emulsion solvent diffusion method using ethyl cellulose as polymer. Drug and polymer in the various quantity (**Table 7**) were dissolved in 1:1 mixture of solvent system of ethanol and dichloromethane. Ethyl cellulose mixture was poured drop wise into 100ml of 0.25% w/v aqueous solution of polyvinyl alcohol (PVA). The solution thus prepared was stirred for 3 h at 600 rpm to allow the volatile solvent to entirely evaporate (**Figure 16**). Filtered microspheres were collected, rinsed with distilled water several times, and dried at room temperature. [20, 21]

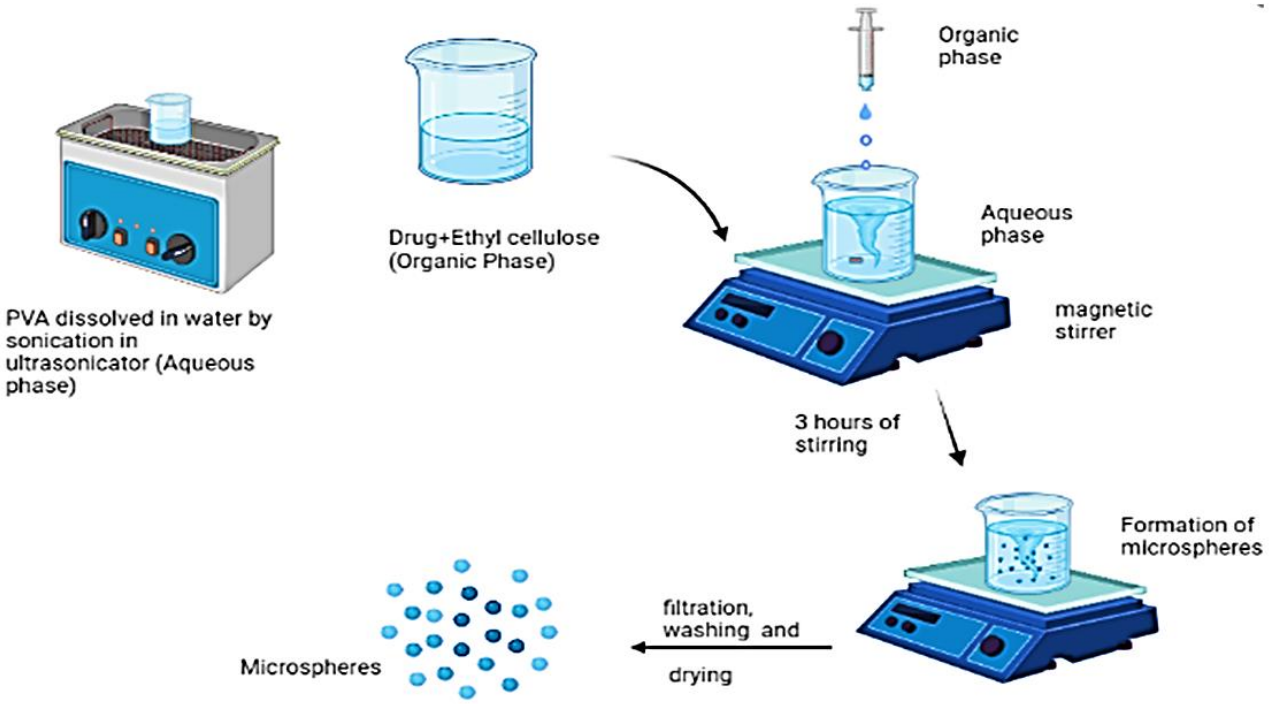

Figure 16. Preparation of floating microspheres

Table 7. Composition of Fenofibrate loaded floating microspheres (Preliminary study)

Batch code	A1	A2	A3	A4
Drug (Fenofibrate) mg	**100**	**100**	**100**	**100**
Ethyl Cellulose (mg)	**100**	**200**	**300**	**400**

4.3 Experimental design

Taguchi Orthogonal array design was employed to formulate the formulation with the help of Design expert software, Version 13, StatEase, USA. On the basis of the preliminary study, batch A2 (1:2) of Ethyl cellulose microspheres was selected as an ideal batch and was kept constant. So, to check the effect of different process variables at their lower and higher-level optimization was carried out by Taguchi design (Table 2). The independent variables chosen were as follows: Polymer concentration (A), stirring rate (B), stirring time (C) and ethanol concentration (D) were taken at various level (Table **8**). The response variables selected were Particle size, % yield, % Drug loading, % Buoyancy and % Drug release. Microspheres were made in the same fashion as previously described, but with different polymer concentrations, stirring speeds and times, and ethanol concentrations.

Table 8. Factors and Levels of Independent variables

Sl no.	Independent variables	Low level	Medium level	High level
01.	Polymer Concentration (mg)	100	200	300
02.	Stirring speed (rpm)	300	600	900
03.	Stirring time (hour)	1	3	5
04.	Ethanol Concentration (ml)	5	10	15

Table 09. Formulation design for Fenofibrate loaded Ethyl Cellulose microspheres as per Taguchi design

Formulation batch	A: Polymer concentration (mg)	B: Stirring speed (rpm)	C: Stirring time (hour)	D: Ethanol concentration (ml)
F1	100	600	3	10
F2	300	600	1	15
F3	100	300	1	5
F4	300	900	3	5
F5	200	300	3	15
F6	100	900	5	15
F7	300	300	5	10
F8	200	900	1	10
F9	200	600	5	5

4.4. Characterization of microspheres

4.4.1 Compatibility studies

FR-IR spectroscopy was used to determine the compatibility of drug and polymer and was carried out using BRUKER ALPHA-2 analyzer, USA. The spectra were recorded between 4000 and 400 cm^{-1} using the potassium bromide disc method. The spectra obtained were compared and interpreted for the functional group peaks. The compatibility of the drug and polymer was determined using FR-IR spectroscopy on a BRUKER ALPHA-2 analyzer. Using the potassium bromide disc method, the spectra were recorded between 4000 and 400 cm^{-1}. For the functional group peaks, the spectra were compared and analyzed.

4.4.2. Determination of percentage yield and drug loading

The microspheres that had developed were collected and weighed, and the % yield of each formulation was estimated using the procedure below.
The developed microspheres were collected and weighed; the percentage yield of each formulation was calculated by using the formula given below

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

10 mg of formulated floating microspheres was taken from each batch, and the drug was extracted using ethanol, the extract was taken in a 100 mL of volumetric flask and the volume was made up using 0.1N HCl. The solution was filtered and after suitable dilution the absorbance was measured at 289 nm spectrophotometrically against appropriate blank.

$$\text{Drug loading } (\%) = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \text{X } 100$$

4.4.3. Particle size analysis

The Particle size of each formulation was determined by dynamic light scattering method using Malvern Mastersizer 3000, UK. In this procedure, a laser beam is sent through the sample, the difference in angular scattered light intensity is measured, and the particle size distribution in the sample is calculated using Mie theory.

4.4.4. Buoyancy

50 mg of microspheres were placed in a beaker containing 100 mL of 0.1N HCl and 0.02% w/v of tween 80. The above mixture was stirred at 100 rpm on a magnetic stirrer for 24 h. After 24 h floating and sedimented microspheres were collected separately and dried in a desiccator. By using the formula given below the floating ability was calculated.

$$\text{Buoyancy } (\%) = \frac{\text{Weight of floating microspheres}}{\text{Initial weight of microspheres}} \text{ X } 100$$

4.4.5. *In-vitro* Drug release studies

The drug release from the floating microspheres was determined using basket type dissolution apparatus (USP type II) 900 mL of 0.1N HCl was used as the dissolution medium, and the temperature was maintained at 37±0.5 °C with a rotation speed of 100 rpm. The floating microspheres equivalent to 100 mg drug was

weighed and filled into a capsule and placed in the basket. The capsule was placed in a non-reacting mesh that had a smaller mesh size than the microspheres. At specified time intervals, 5 mL aliquots were withdrawn and replaced with fresh 0.1N HCl to maintain sink condition. The sample withdrew was analyzed by UV Spectrophotometer at λ_{max} of 289nm after suitable dilution against blank.

4.4.6. Optimization

Using commercially available design expert software, version 13, the response variables were examined using ANOVA. The F-test (p-0.05) was used to evaluate each parameter, and the responses were then subjected to multiple regression analysis to develop polynomial equations. The equations were then used to explain the relationship between the factors and responses.

4.5. Evaluation of optimized microspheres

4.5.1. Scanning electron microscopy (SEM)

SEM was performed to characterize the surface morphology of the optimized formulation, and this was done by using a Scanning electron microscope (Gemini 300, Carl Zeiss, Germany). Prior to analysis, the samples were gold coated to make them electrically conductive. SEM was performed to characterize the surface morphology of the optimized formulation, and this was done by using a Scanning electron microscope (Gemini 300, Carl Zeiss, Germany). Under a microscope, the microspheres were examined, and images were taken.

4.5.2. *In-vitro* drug release kinetics

The release data obtained from in-vitro release study was fitted into different kinetic models like zero order, first order, Higuchi's and Korsmeyer-Peppas model using drug release kinetic software. The release rate constant, slope and regression co-efficient was estimated by incorporating the values of drug release in the models to determine the release mechanism.

Acknowledgements: We the authors acknowledge the management of College of Pharmaceutical Sciences, Dayananda Sagar University for providing the facilities for this research.

Author contributions: Concept – J.L.J.; Design – J.L.J., M.A.R., S.S.R.; Supervision – J.L.J., S.S.R.; Resources – J.L.J., M.A.R.; Materials – J.L.J.; Data Collection and/or Processing – P.S.; Analysis and/or Interpretation – J.L.J., S.S.R.; Literature Search – P.S., S.S.R.; Writing – M.A.R; Critical Reviews –J.L.J., M.A.R., P.S., S.S.R.

Conflict of interest statement: The authors declared no conflict of interest in the manuscript.

REFERENCES

[1] Homayun B, Lin X, Choi HJ. Challenges and Recent Progress in Oral Drug Delivery Systems for Biopharmaceuticals. Pharmaceutics. 2019; 11(3):129. [CrossRef]

[2] Wen H, Jung H, Li X. Drug Delivery Approaches in Addressing Clinical Pharmacology-Related Issues: Opportunities and Challenges. AAPS J. 2015 Nov;17(6):1327-40. [CrossRef]

[3] Pawar VK, Kansal S, Garg G, Awasthi R, Singodia D, Kulkarni GT. Gastroretentive dosage forms: a review with special emphasis on floating drug delivery systems. Drug Deliv. 2011; 18(2): 97-110. [CrossRef]

[4] Hooda A, Nanda A, Jain M, Kumar V, Rathee P. Optimization and evaluation of gastroretentive ranitidine HCl microspheres by using design expert software. Int J Biol Macromol. 2012;51(5):691-700. [CrossRef]

[5] Soppimath KS, Kulkarni AR, Rudzinski WE, Aminabhavi TM. Microspheres as floating drug-delivery systems to increase gastric retention of drugs. Drug Metab Rev. 2001;33(2):149-60. [CrossRef]

[6] Garg RG, Gupta GD. Progress in controlled gastroretentive delivery systems. Trop. J. Pharm. Res. 2008;7(3):1055-66. [CrossRef]

[7] Kapoor D. Formulation, optimization and evaluation of floating microspheres of captopril. Asian J. Biomed. Pharm. Sci. 2012;2(9):1-10.

[8] Kazemi M, Varshosaz J, Tabbakhian M. Preparation and Evaluation of Lipid-Based Liquid Crystalline Formulation of Fenofibrate. Adv Biomed Res. 2018;7:126. [CrossRef]

[9] Pundir R, Chary GH, Dastidar MG. Application of Taguchi method for optimizing the process parameters for the removal of copper and nickel by growing Aspergillus sp. Water Resour Ind. 2018;20:83-92. [CrossRef]

[10] Kotagale NR, Parkhe AP, Jumde AB, Khandelwal HM, Umekar MJ. Ranitidine hydrochloride-loaded ethyl cellulose and eudragit Rs 100 buoyant microspheres: Effect of ph modifiers. Indian J. Pharm. Sci. 2011;73(6):626. [CrossRef]

[11] Junyaprasert VB, Pornsuwannapha S. Floating properties and release characteristics of hollow microspheres of acyclovir. Drug Delivery. 2008;15(5):331-41. [CrossRef]

[12] Avachat AM, Bornare PN, Dash RR. Sustained release microspheres of ropinirole hydrochloride: effect of process parameters. Acta Pharm. 2011;61(4):363-76. [CrossRef]

[13] Patel A. Formulatıon and evaluatıon of floatıng mıcrospheres for oedema. Int. J. Pharm. Sci. Res. 2012;3(12):4997. [CrossRef]

[14] Nepal PR, Chun MK, Choi HK. Preparation of floating microspheres for fish farming. Int J Pharm. 2007;341(1-2):85-90. [CrossRef]

[15] Pandey N, Sah AN, Mahara K. Formulation and evaluation of floating microspheres of nateglinide. Int. J. Pharma Sci. Res.2016;7(11):453-64.

[16] Patel AK, Mishra MK, Gupta J, Ghoshal S, Gupta R, Kushwaha K. Guar Gum-based floating microspheres of repaglinide using 32 factorial design: fabrication, optimization, characterization, and in vivo buoyancy behavior in albino rats. Assay Drug Dev Technol. 2021;19(2):63-74. [CrossRef]

[17] Dey S, Pramanik S, Malgope A. Formulation and optimization of sustained release stavudine microspheres using response surface methodology. Int Sch Res Notices. 2011;2011. [CrossRef]

[18] Fentie M, Belete A, Gebre-Mariam T. Formulation and optimization of Controlled release Floating Microspheres of Furosemide from Ethylcellulose and Hydroxypropyl methylcellulose polymer blends. Int J Pharm Sci Res. 2014;5(1):70-82. [CrossRef]

[19] Bhardwaj P, Chaurasia D, Singh R, Swarup A. Development and characterization of novel site specific hollow floating microspheres bearing 5-Fu for stomach targeting. Sci. World J. 2014;2014. [CrossRef]

[20] Rikita A, Anup O, Himanusu C, Kumud U. Formulation and evaluation of floating microsphere of levofloxacin. Int. Res. J. Pharm. 2018;9(7):186-91. [CrossRef]

[21] Pandya N, Pandya M, Bhaskar VH. Preparation and in vitro Characterization of Porous Carrier-Based Glipizide Floating Microspheres for Gastric Delivery. J Young Pharm. 2011;3(2):97-104. [CrossRef]