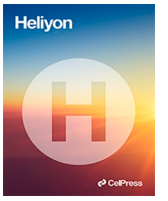
Heliyon 10 (2024) e24301

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Enhanced gastric residence time of acyclovir by floating raft formulation using box-behnken design

Rajalakshmi Munusamy [a], Sangeetha Shanmugasundharam [a,*]

[a] Department of Pharmaceutics, SRM College of Pharmacy, SRMIST, Kattankulathur, Chengalpattu, 603203, India

ARTICLE INFO

Keywords:
Acyclovir
Raft forming drug delivery system
Box behnken design
Optimization
Gastric residence time

ABSTRACT

This research paper reports enhancing Acyclovir's gastric residence time by implementing a raft-forming drug delivery system. Because acyclovir is a narrow absorption window drug, it has a poor bioavailability of 10–20 % and a short half-life ($t_{1/2}$) of 2.5 h. The guar gum and GMS-based floating raft formulation retain the drug in the stomach for an extended period by enhancing GRT. The Box-Behnken design is used to optimize the amount of guar gum, glyceryl monostearate, and calcium carbonate and to study how they affect the in vitro gelation time, viscosity, and in vitro drug release. The ratio of drug and excipients in guar gum (1:0.5), GMS (1:1.25) based FRF suspension containing sodium citrate (0.25 %), carbopol (0.1 %), and calcium carbonate (1:1.5). Seventeen runs were developed through the Box-Behnken design to study all the optimal interactions between variables and responses through a polynomial equation. The optimized formulation is then characterized using various physicochemical tests such as rheological analysis, in vitro drug release, kinetic drug release, and in vitro permeation studies. The in vitro gelation time, viscosity, and in vitro drug release time of optimized FRF are 12 s, 1090 cps, and 88 % at 24 h, respectively. The flux and permeability coefficient of the optimized batch have a higher value indicating higher permeability of acyclovir. The FRF follows non-fickian diffusion as a drug release mechanism. The results show that the raft-forming drug delivery system significantly enhances the absorption of Acyclovir by prolonging drug release and also improving its gastric residence time in the stomach. This research contributes to the field of drug delivery systems by providing a novel approach for improving the therapeutic efficacy of acyclovir and potentially other drugs with similar characteristics.

1. Introduction

Acyclovir [9-(2-hydroxyethoxymethyl) guanine; Zovirax] is primarily employed in first-line therapy for herpes virus and varicella-zoster virus and comes under the classification of BCS class III. The affinity of acyclovir for the enzyme thymidine kinase encoded in HSV and VZV is highly selective, resulting in increased inhibitory activity on the virus. The oral absorption of acyclovir is mainly in the upper part of the GIT; because acyclovir is a narrow absorption window drug, it has poor bioavailability of 10–20 % and a short half-life ($t_{1/2}$) of 2.5 h. Nevertheless, the drug has a low gastric retention time in the stomach, and when it reaches the lower part of the GIT, it is unavailable or poorly available for absorption of the drug. Due to its short half-life, poor absorption, and poor bioavailability, it is often administered in higher doses to maintain therapeutic efficacy. Overdose can cause the general side effects of anaphylaxis,

* Corresponding author.
E-mail address: sangeets2@srmist.edu.in (S. Shanmugasundharam).

https://doi.org/10.1016/j.heliyon.2024.e24301
Received 3 June 2023; Received in revised form 4 January 2024; Accepted 5 January 2024
Available online 7 January 2024

Abbreviation

GRDDS	Gastro Retentive Drug Delivery System
RFDDS	Raft Forming Drug Delivery System
GERD	Gastro Oesophageal Reflux Disease
GIT	Gastro-Intestinal Tract
GRT	Gastric Residence Time
FRF	Floating Raft Formulation
GMS	Glyceryl Mono Stearate
XRD	X-Ray diffraction analysis
SEM	Scanning Electron Microscopy
BBD	Box behnken design

angioedema, fever, headache, peripheral edema, and gastrointestinal disturbances like diarrhea and nausea [1–4].

Acyclovir can be developed as a gastroretentive drug delivery system that retains the drug in the stomach for an extended period and improves the bioavailability of drugs with a narrow absorption window in the upper part of the GIT [5]. In this, various approaches are applied to prolong the gastric residence time of the drug, such as the mucoadhesive system, low density (floating), expandable (swelling), and high density (sinking) [6,7].

Raft-forming drug delivery systems are an advanced, revolutionary technique in oral controlled and targeted drug delivery. This effervescent low-density floating system develops a floating layer (raft) with in-situ gelling properties that is intact and enables the layer to sustain for more than 24 h in the stomach [8,9].

A floating layer can be developed from the formulation (suspension, emulsion, or tablet) in contact with gastric fluid, which involves sol-gel conversion and releases carbon dioxide, wherein each portion of the liquid swells and forms a continuous, cohesive gel layer called a raft. Without affecting the gastric emptying rate, this raft layer remains floating on the gastric fluid for an extended period due to the low density created by the release of CO_2 [10,11].

This FRF design aims to enhance the bioavailability of acyclovir by improving the permeability and gastric residence time through guar gum and glyceryl monostearate (GMS) based floating raft formulation (FRF) using the box behnken design. The advantages of the floating raft are that it may reduce the dosing frequency, improve efficacy, and improve patient compliance, and the developed formulation has reproducibility (simple manufacturing method). Raft-forming polymers (Guar gum), gel-forming substances (Carbopol), and effervescent agents (Calcium carbonate) are the key ingredients to produce a raft [12,13]. Guar gum is less expensive, nontoxic, chemically inert, biodegradable, and readily available. Also, it has a potential effect on the pharmaceutical field, acting as a raft-forming polymer as well as an emulsifier, which improves the permeation rate of the drug; it's due to the intrinsic properties that develop as gels when in contact with an acidic medium [14]. Glyceryl monostearate is a lipid used to retard the drug release in the formulation by the drug is incorporated or coated with the glyceryl monostearate [15]. Calcium carbonate can accelerate floatation by acting as an effervescent agent when it comes in contact with the acidic, which releases the carbon dioxide. The generated carbon dioxide is prone to becoming trapped within the raft gel, consequently enhancing the buoyancy of the raft. Gel-forming substances like carbopol are used to strengthen and enhance the floating capability of the formulation because gels remain intact within the stomach for several hours resulting in prolonged drug delivery in the upper part of gastrointestinal tract [16] Sodium citrate is a complexing agent [17] used to prevent premature gelation of the formulation during storage. Box Behnken design (BBD) optimizes the critical ingredients in raft-forming drug delivery; because BBD has a balanced factorial design that reduces the experimental time, cost, and noise sensitivity and identifies the optimal condition for a process or system [18,19].

2. Materials and method

Acyclovir was gift sample from sterile gene laboratory (Pondicherry, India). Guar gum, calcium carbonate and Glyceryl monostearate (GMS, lipid) was provided by Madras Pharmaceuticals (chennai, India) sodium citrate and Carbopol 974p was purchased from SISCO Research laboratory PVT LTD.

Table 1
Variables and their levels in Experimental design.

Independent variables	Levels		
	−1	0	1
Guar gum (X1)	0.5	1	1.5
Calcium carbonate (X2)	1	1.25	1.5
Lipid (X3)	1	1.25	1.5
Dependent variables			**Constraints**
***In-vitro* gelation time**			Minimum
Viscosity			Minimum
***In-vitro* drug release study**			Maximum

2.1. Method

Step 1. Preparation of Floating Raft Formulation:

The polymers of guar gum and GMS-based FRF formulation have calcium carbonate as an effervescent agent, sodium citrate as a complexing agent (0.25 %), and carbopol (0.1 %) as a raft-strengthening agent. The guar gum was dispersed in deionized water containing 0.25 % sodium citrate and heated up to 90 °C under continuous stirring until a homogeneous solution was formed [20,21]. Totally 17 formulations were developed; of those, four were without lipids and others with lipids. Table 3 depicts the list of excipients used in FRF formulation. The process of floating raft formulation is mentioned in Fig. 1.

Step 2. Incorporation of a drug into lipids

Acyclovir was incorporated with glyceryl monostearate (GMS) to retard the drug release rate in the floating raft formulation. The ratio of drug and lipid is 1:1, 1:1.25, and 1:1.5, respectively. In a clean china dish, GMS was melted at 55 °C. After which, the calculated amount of acyclovir was dispersed in molten lipids.

Step 3. Incorporation of Molten Lipid in Floating Raft Formulation:

The ratio of drug to polymer was 1:1, 1:1.25, and 1:1.5. The prepared floating raft formulation was heated up to the same temperature as molten lipid and then added to the acyclovir lipid dispersion. Mix the dispersion using a high-speed homogenizer at 4500 RPM for 10–15 min until a stable emulsion is obtained. Calcium carbonate dispersion was added to the prepared emulsion under homogenization [22].

2.2. Optimization of floating raft formulation by the box-behnken method (BBD)

The effect of raft development and drug release depends on the ratio of polymer, lipid, and effervescent agents in the formulation. BBD performs extensive optimization on these ratios by building polynomial models through experiments with three factors at three levels. A total of 17 run formulations were prepared as per BBD, for investigating the quadratic response surface and constructing a second-order polynomial model using Design-Expert software (Trial Version 12, Stat-Ease Inc., MN). The design consists of a set of midpoints lying at each edge of the multidimensional cube and replicated center points, which are used to analyze the main effects, quadratic effects, and interaction effects of the formulation [23]. The three different levels of the independent formulations are in the ratio of 0.5, 1, and 1.5 of guar gum (X_1), 1, 1.25, and 1.5 of both calcium carbonate (X_2) and lipid (X_3). The experimental design of variables and their levels are shown in Table 1, and different runs of FRF are shown in Table 2.

Stepwise regression analysis of the non-linear quadric model was utilized to create polynomial equations.

$$Y = b_0+b_1X_1+ b_2X_2+b_3X_3+b_4X_1X_2+b_5X_2X_3+b_6+X_1X_3+b_7X_1^2+b_8X_2^2+b_9X_3^2$$

Where Y is response of dependent variables (*in-vitro* gelation time (Y1), *in-vitro* drug release study (Y2), and Viscosity (Y3)), $b_{0\ is}$ arithmetic mean response and b_1, b_2, and b_3 are the estimated coefficient factor of main effects are X_1 X_2 and X_3 respectively. The b_4, b_5, and b_6 are interaction effects between X_1X_2, $X_2X_3$and X_1X_3, b_i (i = 7, 8, or 9) represents the quadratic effects are X_1^2, X_2^2, and X_3^2.

Table 2
Box behnken experimental design for floating raft formulation.

Run	Factor 1	Factor 2	Factor 3
	Guar gum, X_1	Calcium Carbonate, X_2	Lipid, X_3
FRF 1	1.5	1	1.25
FRF 2	0.5	1.25	1
FRF 3	1.5	1.5	1.25
FRF 4	0.5	1	1.25
FRF 5	1	1.25	1.25
FRF 6	1	1.25	1.25
FRF 7	1	1.25	1.25
FRF 8	0.5	1.25	1.5
FRF 9	1	1.25	1.25
FRF 10	1	1.5	1.5
FRF 11	0.5	1.5	1.25
FRF 12	1	1.5	1
FRF 13	1	1	1
FRF 14	1	1	1.5
FRF 15	1	1.25	1.25
FRF 16	1.5	1.25	1
FRF 17	1.5	1.25	1.5

Table 3
Excipients and their ratio in Acyclovir FRF Formulation.

S.No	Ingredients	Application	Ratio (drug:excipient)
1	Guar gum	Polymer, Raft forming agent, Emulsifier	1:0.5, 1:1, 1:1.5
2	Calcium carbonate	Effervescent agent,	1:1, 1:1.25, 1:1.5
3	GMS	Lipid, To retard drug release in FRF	1:1, 1:1.25, 1:1.5
4	Sodium citrate	Complexing agent	0.25 %
5	Carbopol	Gel forming agent, Permeation enhancer	0.1 %

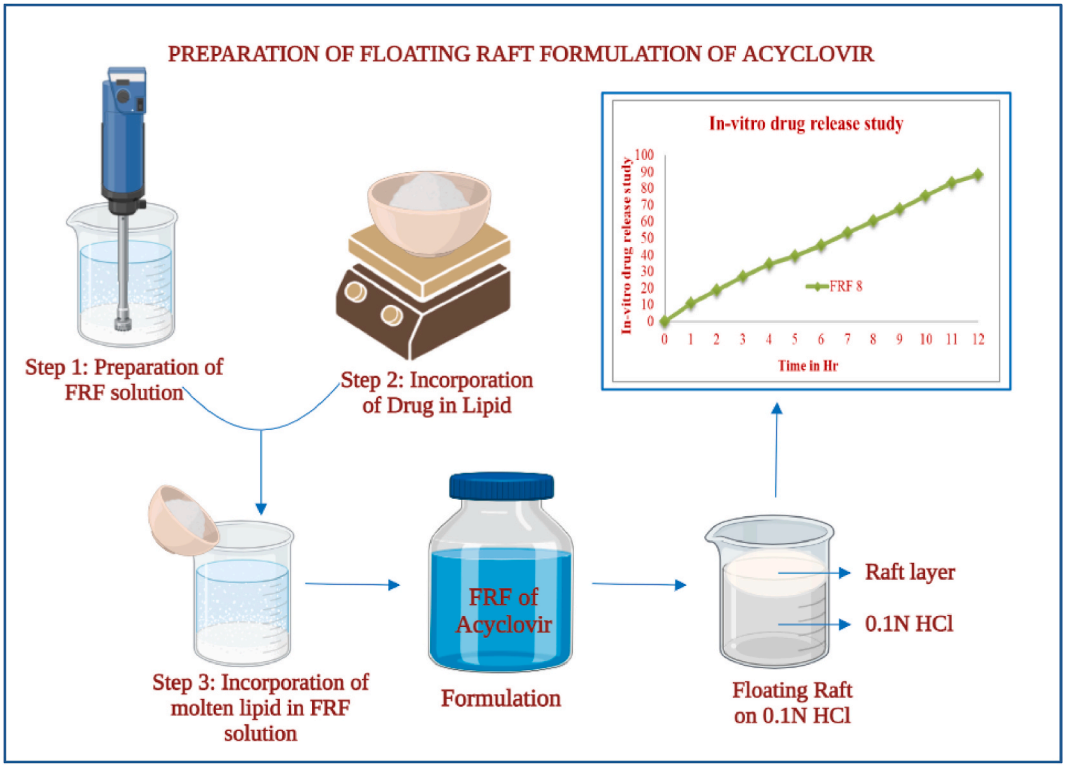


Fig. 1. Illustration of floating raft formulation.

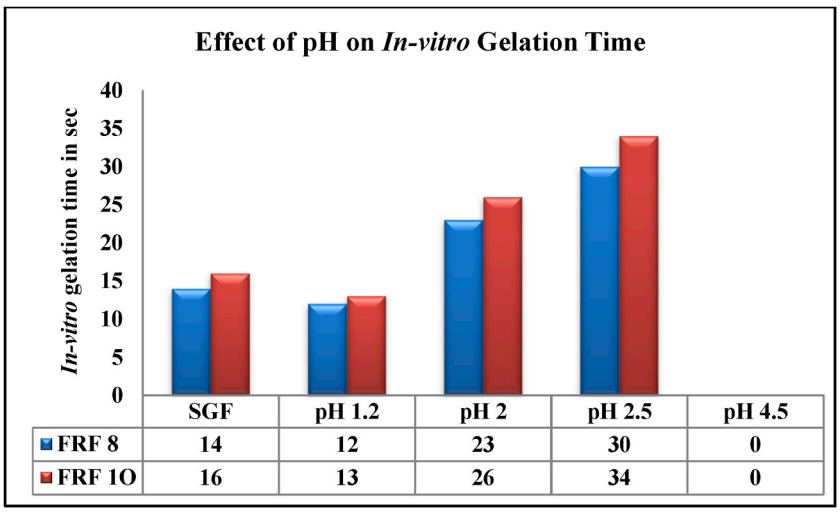


Fig. 2. Effect of pH on *In-vitro* Gelation Time.

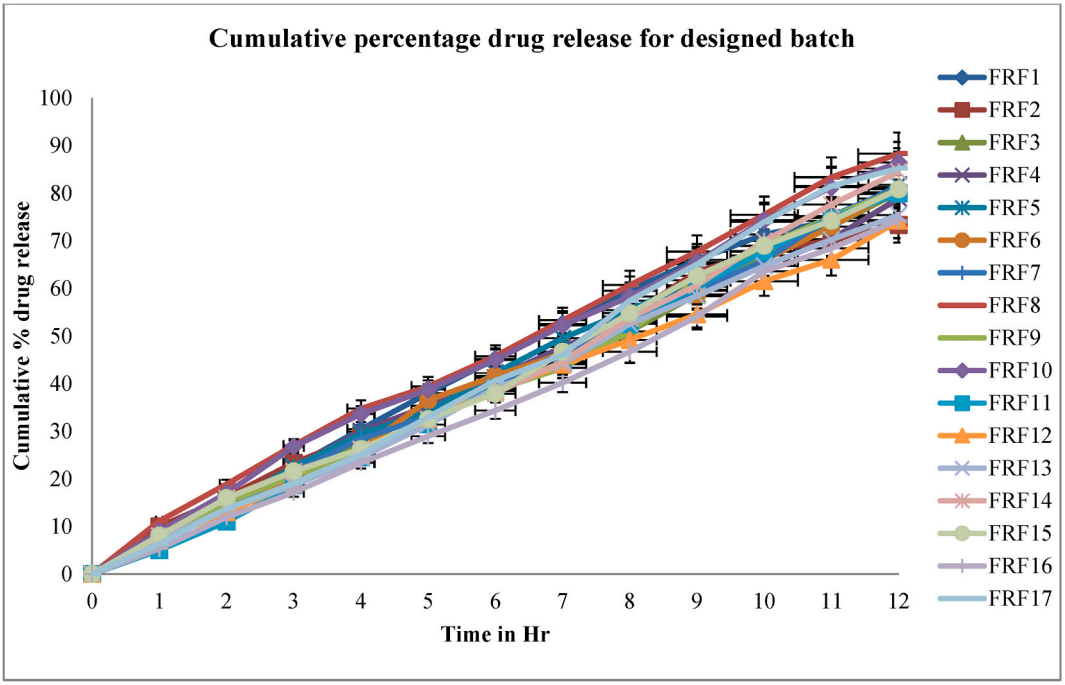


Fig. 3. *In-vitro* drug release study of designed batch.

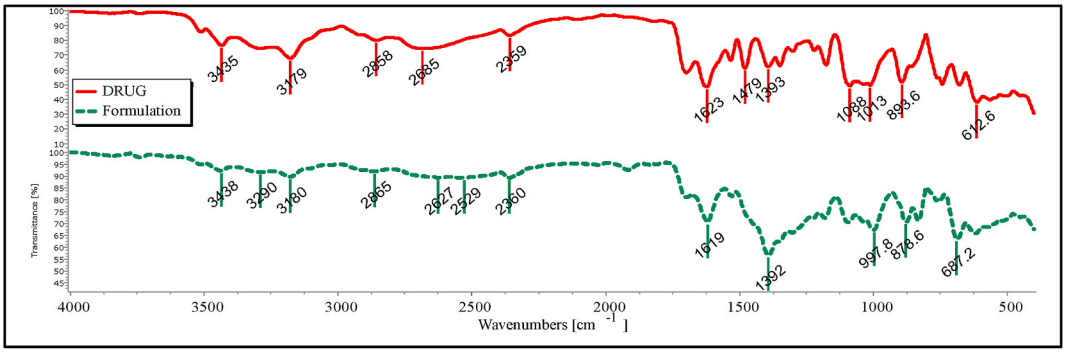


Fig. 4. FTIR spectra of pure drug and FRF (formulation).

3. Evaluation of floating raft formulation

3.1. X-ray diffraction

The crystallinity of the drug, drug-loaded with lipid and FRF (the floating layer was dried), was analyzed using XRD. The diffractograms of a pure drug, drug loaded with lipids, and the floating raft formulation (the floating raft layer was dried) were obtained. The instrument was set by Cu-radiation (λ 1.534 Å) and applied 40 Kv of voltage [24].

3.2. Particle size analysis

The zeta potential analysis (Malvern Zetasizer) surface charge and aggregation behavior of the pure drug, drug-loaded lipid, and formulated FRF were determined. The samples were dispersed in water and sonicated for about 30 min for analysis, later, particle size and zeta potential were measured [25].

3.3. Effect of pH

The pH of each formulation should be within the range (pH 7 to 7.4) and adjusted with an alkalizer. The effect of raft development

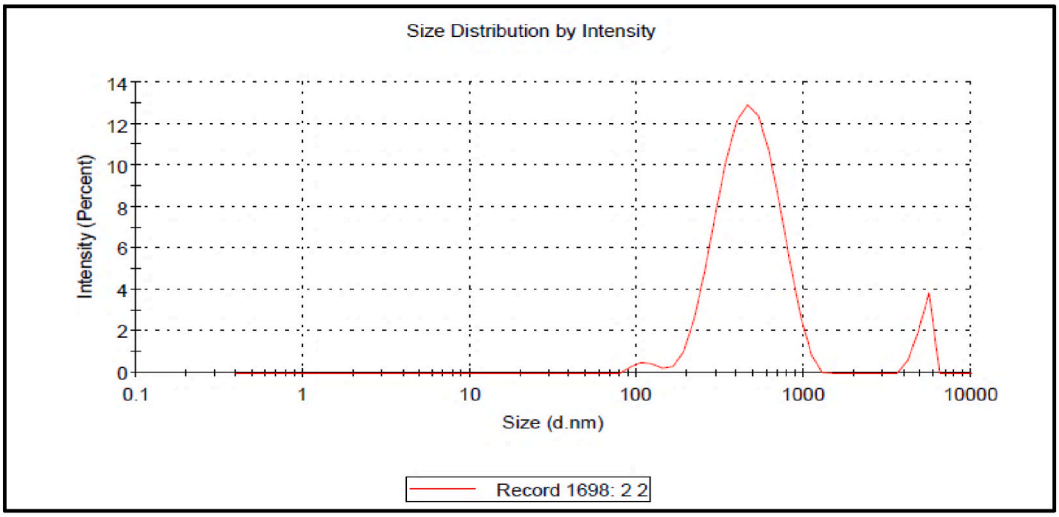


Fig. 5. Particle size of Drug loaded in Lipid.

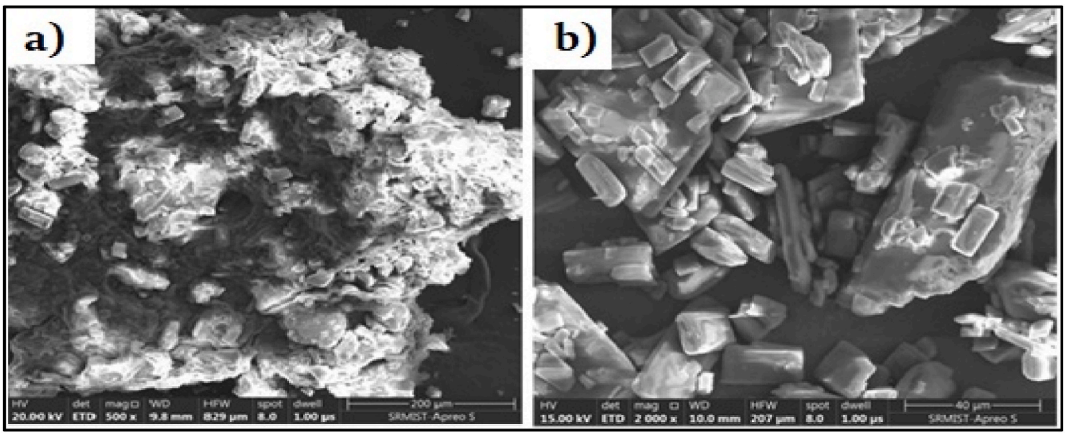


Fig. 6. SEM image of a) Pure drug, and b) drug loaded with lipid.

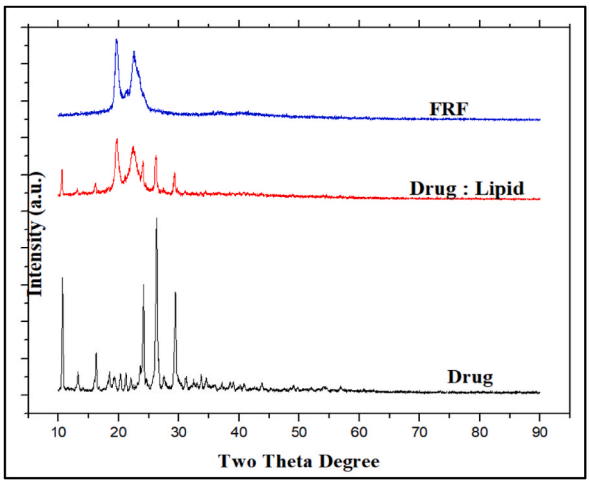


Fig. 7. XRD Spectra of Drug, Drug loaded Lipid and FRF.

was determined with different buffers like pH 1.2, 2, 2.5, 4.5, and SGF. Prepared formulations were introduced into the selected buffer at room temperature, and the final pH was noted. Simulated gastric fluid (pH 1.2) was prepared with 3.2 g of pepsin, 2 g of sodium chloride, and 7 ml of HCl added to 1000 ml of distilled water [20].

3.4. Measurement of viscosity

The rotating viscometer determines the viscosity by torque percentage. A sample determines the appropriate spindle number and rotational speed. Adjust the groove mark on the viscometer until the spindle dips into the sample. Turn on the motor and allow the spindle to rotate until a constant reading is obtained. The torque percentage should be between 10 and 100 %. Change the spindle number and rotational speed if the torque percentage exceeds or falls below the limit [10].

3.5. In-vitro gelation time

10 ml of the formulation was added to a 250 ml beaker, which contains 100 ml of buffer (pH 1.2), to determine the in-vitro gelation time. The time needed to form a raft on the solution and clear the lower part of the beaker is known as in-vitro gelation time [26].

3.6. In-vitro floating lag time

10 ml of the formulation was added to a 250 ml beaker, which contains 100 ml of buffer pH 1.2, to determine the floating time. This is described by the extensive-time period during which the raft remains afloat on the liquid [27].

3.7. Raft volume

The empty beaker was weighed after it had dried completely and written as W1. 100 ml of buffer pH 1.2 (raft-developing liquid) was transferred in a beaker; note the level of the liquid; it was then weighed and written as W2. In this beaker, 10 ml of the formulation was added. Then allow it to develop a raft and keep it aside for a few minutes. Following that, the raft was collected on butter paper and dried with paper towels before being weighed and recorded as W3. Purified water was filled up to the mark made in the same beaker used for raft development while 100 ml of raft-developing liquid was added. In this dried raft, weight was taken and written as W4, with all the collected data on raft volume estimated by the below formula [28].

Raft Volume = (W4–W1)-(W2–W1–W3)

3.8. Raft weight

For this test, 100 ml of buffer pH 1.2 was taken in a 250 ml beaker. In this, 10 ml of the formulation was poured, and raft layers developed uniformly. After the complete development of the raft, the beaker remains were kept to one side for 30 min, and the leftover liquid in the beaker was decanted. The raft was collected from a beaker and transferred to the butter paper; then excess liquid in the raft was removed by using a paper towel, and the raft dried for 2 h [29].

3.9. In vitro release study

The USP type II dissolution apparatus determined the in-vitro drug release data. The paddle shaft was rotated at 25 rpm, and the chamber was maintained at 37C ± 2. For this study, 900 ml of pH 1.2 buffer was taken in a basket. 10 ml of the formulation was placed in the dissolution testing basket, and the raft was left to develop uniformly. Then the paddle began to rotate at 25 rpm, and from the basket, 5 ml of solution was taken and filtered. From the filtrate, 1 ml was pipetted out into 10 ml standard flasks, and the volume was made up to the mark at every 1-h time interval, and the volume was filled up to the level with buffer pH 1.2. Replace the volume with 5 ml of pH 1.2 buffer. In a UV–visible double-beam spectrophotometer, the absorbance was analyzed at 265 nm to get the concentration by applying the calibration factor**.** [21,30].

The release profile of acyclovir was determined with different buffers; because presence of food content changes the stomach pH; this may influence development of raft in human stomach. To replicate the same condition the drug release study performed at different medium (pH 1.2, 2, 2.5, 4.5).

3.10. Release kinetic study

The kinetics of drug release and its mechanism can be studied by applying the in vitro drug release data to various kinetic models like the zero-order, first-order, Higuchi's, and Korsmeyer-Peppas models. The correlation coefficient of linear curves obtained from the regression analysis of the above model [31].

3.11. In-vitro permeation study

The in-vitro permeation study for the optimized acyclovir FRF batch was performed using Franz diffusion. Dialysis membrane 70 was previously soaked in 0.1 N HCl, placed between the donor and receptor compartments of the diffusion cell. The receptor chamber was filled with freshly prepared 0.1 N HCl (pH 1.2). The acyclovir FRF formulation (the previously developed raft layer) was placed in the donor compartment. The dissolution medium was continuously stirred with a magnetic stirrer (rise bead) at 20 rpm effectively mix the receptor fluid, and to uphold a consistent concentration level throughout the entire receptor chamber. 1 ml of samples was withdrawn from the receptor compartment at suitable time intervals (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 h) and replaced with 1 ml of 0.1 N HCl to maintain sink condition. The collected samples were filtered and diluted with medium, and then the drug content was analyzed using a UV–visible spectrophotometer at 254 nm. The cumulative amount of drug permeated through the dialysis membrane, flux, and permeation coefficient were calculated [32].

4. Results and discussion

4.1. Characterization of acyclovir and formulation

The FTIR spectra of the Acyclovir and FRF formulation shown in Fig. 4. The acyclovir showed peaks at 3512.37 cm−1 (OH group stretching), 3288 cm−1 (NH_2 group stretching) 1699.24 cm−1(C═O group stretching), 1479.40 cm−1 (C═N group stretching) and 1179.58 (C–N group stretching) assures no chemical interaction between drug and polymer.

The particle sizes of pure drug and drug loaded with lipids were determined with the Malvern zeta sizer and SEM analysis depicted in Figs. 5 and 6 (a, b) respectively. The pure drug has an average particle size of 1501 d nm; after being loaded with lipid, the size was 493.4 d nm. The drug-loaded lipids with severe negative/positive surface charges are stable. A flat-out zeta possible estimation of ±30 mV is an overall sign that the colloidal solutions are highly stable. The drug loaded with lipids had a mean size of around 500 nm and a zeta potential of −14.4 mV. These findings suggest that a pure drug stabilized with lipid coating carried negative charges, and the drug was stable due to the powerful repulsion between the drug particles due to lipid coating. The formulation's SEM image showed the drug's particle size and loaded with lipid were 200 μm and 40 μm, respectively.

The XRD of pure drug, drug incorporated in lipid, and FRF 8 was performed and shown in Fig. 7. A pure drug has many lines of diffraction, which indicates that the distribution of the drug cannot be uniform. The drug was coated with lipids, and the formulations showed two different peaks, and the intensity of the peaks was less as compared to the peaks of the drug, indicating the uniform distribution of the drug.

4.2. Effect of pH on in-vitro gelation time

The effect of raft formation at different pH was analyzed to mimic the stomach pH at fasting and fed states. The in-vitro gelation time is affected by the environmental pH of the medium. pH at 1.2, SGF gelation occurs immediately, and at pH 2 and 2.5, it takes a few seconds for sol-gel conversion, But at pH 4.5, gelation doesn't take place, which confirms that the pH of the medium influences the rate and effect of the in-vitro gelation time

Mentioned in Fig. 2.

4.3. Effect of viscosity on FRF

A higher concentration of guar gum increases the viscosity of the final product, which results in the formulation not having to pour consistency and delayed *in*-vitro gelation time. This is due to interaction and entanglement between polymer chains. It was also observed that higher concentrations of the polymer exhibited shear thinning behavior, as had been reported. By reducing the amount of guar gum, the FRF could achieve its ideal viscosity. The viscosity of the designed batches is between 1090 and 1480; from this, FRF 8 and FRF 10 have adorable viscosities of 1090 and 1150, respectively.

4.4. The in-vitro gelation time and total floating time on FRF

When the formulation comes into contact with an acidic environment, a gel forms because the dispersed calcium carbonate releases carbon dioxide. This starts with *in-situ* gelation, which creates a cohesive gel layer (a "floating raft") on the medium. The *in-vitro* gelation time of the optimized formulation was floated within 12–34 s. The FRF 8 and FRF 10 have better effects on gelation times of 12 and 13 s, respectively. The total floating capacity of the designed formulation has exhibited more or less 12.

4.5. Raft weight and volume of FRF

The raft weight and raft volume of all optimized batches are 2.052–2.772 g and 2.199–3.281 ml, respectively. The raft weight and volume depend on the amount of carbopol and polymer present in the formulation. The floating raft layer may get disturbed by the presence of food in the stomach or while drinking water, which affects the total floating capacity of FRF and also makes the rate of gelation formation slower with disrupted parts of the gel due to the consistency of the suspension. To avoid this situation, the raft weight and volume should be higher with a low amount of polymer. Carbopol is a gel-forming agent that assures the optimum raft

weight and raft volume. Formulations were developing intact gel patches only at pH 1.2 and 2.5, this is essential for loner floating capacity and to retain the drug for a longer period of time in the stomach.

4.6. In vitro drug release study

The in vitro drug release study concluded that the acyclovir FRF-optimized formulation effectively releases the drug at pH 1.2 and 2 than pH 2.5 and 4.5. The release profile of acyclovir extended the release of the drug for the next 12 h (88 % of the drug was released sustainably). This is due to the lipid present in the FRF retaining the drug for a longer period and also extending the drug release maximum of 74–88 % at 12 h, as depicted in Fig. 3. All the designed batches succeeded in forming gelation and floated upon contacting dissolution medium (pH 1.2, 2, 2.5, and SGF), and at pH 2.5, gelation occurs, but the drug fails to release the drug in a sustained manner, and 4.5 gelations didn't occur, and the drug release was very poor, which indicates that the optimized acyclovir FRF releases the drug for a longer time in the stomach at pH 1.2 and 2. The results of all designed FRF batches are depicted in Table 4.

4.7. Statistical analysis of experimental data by Design-Expert software

The results of the optimized batch were analyzed using Design-Expert software. The selected independent variables include the amount of guar gum, calcium carbonate, and lipid (GMS) influencing the response of the in vitro gelation time, viscosity, and in vitro drug release study. The results of the statistical analysis of the design batches are depicted in Table 5. The polynomial equations of the statistical model were established by ANOVA, R2value, p-value, F-value, and correlation coefficient generated in Design Expert software. The interaction effects of two independent variables on dependent variables, or responses, were graphically represented through response surface plots. The contour plot and 3-D response surface plot of various responses of in-vitro gelation time, viscosity, and *in-vitro* drug release study were depicted in Fig. 8 (a, b), 9 (a, b), and 10 (a, b). This parameter helps to observe the qualitative effect of each factor on the response (see Fig. 9).

4.8. Response 1 (Y_1): In vitro gelation time

The in vitro gelation time ranged from 12 s (FRF8) to 35 s (FRF1) for various formulations. The regression analysis proved the significant effect of independent variables on the quantity of guar gum and calcium carbonate on the gelation time. The effect can be explained through the following polynomial equation:

$$Y_1 = 23.4–0.625X_1+9.25X_2\text{-}1.13X_3\text{-}0.25X_1X_2\text{-}0.5X_2X_3\text{-}0.25 \times 1 \times {}_3+0.8X_1^2\text{-}0.95X_2^2\text{-}2.2 \times {}^2_3$$

The equation indicates that the responses increase with a higher amount of calcium carbonate and a lower amount of guar gum, and the R2 value of the above equation is 0.9411, which indicates a good fit for the model.

4.9. Response 2 (Y_2): viscosity

The viscosity of all the designed batches ranges from 1090 (FRF 8) to 1480 (FRF 1), and the regression analysis assures that the significant effect of independent factors (guar gum and calcium carbonate) influences the viscosity of the formulation. When the concentration of polymers and lipids is higher, the viscosity increases. The interaction effect of guar gum and calcium carbonate on viscosity is explained through the following regression analysis of the polynomial equation. The positive value assures that the responses increase with a higher quantity of guar gum and viscosity. The R2 value of response Y2 is around 0.9945, which indicates the model is fit for the responses.

$$Y_2 = 1234 + 180X_1\text{-}1.25X_2\text{-}1.25X_3+2.54 \times 1 \times {}_2+12.50 \times 2 \times {}_3+0.125 \times 1 \times {}_3+53 \times {}^2_1+0.5 \times {}^2_2+0.5 \times {}^2_3$$

4.10. Response 3 (Y_3): in-vitro drug release study

The in vitro drug release study of all batches has a value ranging from 74.23 to 86.46 %. The regression analysis confirms the significant effect of independent variables on the dependent variable (response 3). The in vitro drug release time was enhanced with a higher amount of guar gum and lipid. The effect of interaction between response 3 and the independent variable is explained through the following polynomial equation. The R2 value of Response 3 is 0.9334, which assures the responses fit the selected design. $Y_3 = 81.1 + 0.135X_1 + 0.265X_2+5.63X_3\text{-}0.1675 \times 1 \times {}_2+0.5225X_2X_3\text{-}0.7875X_1X_3\text{-}0.8653X_1^2\text{-}0.5103X_2^2\text{-}0.4302 \times {}^2_3$

4.11. Optimization and validation

The optimum formulation was based on the set criteria of minimum in vitro gelation time, minimum viscosity, and maximum in vitro drug release study (see Fig. 10). Therefore, the predicted levels of responses with a new formulation of FRF were prepared to confirm the validity of the optimization procedure. The composition of the optimized formulation was 0.5 % guar gum, 1.5 % calcium carbonate, and 1.5 % lipid (GMS), which satisfy the requirements. The responses of the optimized batch have 12 s of in-vitro gelation

Table 4
Results of different evaluation parameters of FRF.

Run	pH	Floating Time (Hr)	Raft Weight (gm)	Raft Volume (ml)	*In-vitro* gelation time (sec)	Viscosity (cps)	Cumulative % drug release
FRF 1	7.40 ± 0.3	>12	2.311 ± 0.4	2.681 ± 0.8	35 ± 2	1480 ± 10	79.84 ± 0.5
FRF 2	7.40 ± 0.2	>12	2.129 ± 0.8	2.549 ± 0.5	25 ± 3	1130 ± 20	74.35 ± 0.7
FRF 3	7.40 ± 0.5	>12	2.078 ± 0.2	2.238 ± 0.4	22 ± 4	1460 ± 10	80.21 ± 0.1
FRF 4	7.40 ± 0.4	>12	2.172 ± 0.9	2.912 ± 0.8	34 ± 4	1120 ± 30	78.92 ± 0.3
FRF 5	7.40 ± 0.7	>12	2.062 ± 0.7	2.822 ± 0.2	24 ± 2	1240 ± 10	81.79 ± 0.4
FRF 6	7.40 ± 0.3	>12	2.091 ± 0.1	2.610 ± 0.3	23 ± 3	1230 ± 30	80.84 ± 0.3
FRF 7	7.40 ± 0.6	>12	2.173 ± 0.8	2.57 ± 0.28	24 ± 4	1240 ± 20	81.23 ± 0.4
FRF 8	7.40 ± 0.2	>12	2.772 ± 0.9	3.281 ± 0.8	12 ± 2	1090 ± 10	88.32 ± 0.5
FRF 9	7.40 ± 0.3	>12	2.052 ± 0.6	2.199 ± 0.5	23 ± 2	1230 ± 30	80.84 ± 0.4
FRF 10	7.40 ± 0.5	>12	2.521 ± 0.7	3.278 ± 0.4	13 ± 2	1150 ± 20	86.46 ± 0.7
FRF 11	7.40 ± 0.7	>12	2.173 ± 0.8	2.972 ± 0.8	22 ± 3	1090 ± 20	79.96 ± 0.5
FRF 12	7.40 ± 0.6	>12	2.362 ± 0.9	2.862 ± 0.2	23 ± 3	1240 ± 20	74.23 ± 0.7
FRF 13	7.40 ± 0.5	>12	2.052 ± 0.7	2.691 ± 0.3	28 ± 4	1220 ± 20	75.45 ± 0.7
FRF 14	7.40 ± 0.5	>12	2.391 ± 0.5	3.057 ± 0.2	27 ± 4	1230 ± 20	84.53 ± 0.8
FRF 15	7.40 ± 0.3	>12	2.313 ± 0.8	2.681 ± 0.8	23 ± 3	1230 ± 30	80.84 ± 0.5
FRF 16	7.40 ± 0.5	>12	2.067 ± 0.1	2.499 ± 0.5	23 ± 3	146,020	74.35 ± 0.4
FRF 17	7.40 ± 0.4	>12	2.452 ± 0.3	3.078 ± 0.4	18 ± 4	1470 ± 20	85.23 ± 0.4

Table 5
Statistical analysis of experimental data by box behnken method.

Factor	X1			X2			X3		
	p-value	F-value	Coefficient	p-value	F-value	Coefficient	p-value	F-value	Coefficient
Model/Intercept	0.0016	12.43	23.4	<0.0001	141.4	1234	<0.0001	116.94	81.11
X Guar gum	0.5102	0.4813	0.625	<0.0001	1213.65	180	0.4714	0.5793	0.135
B-Calcium Carbonate	<0.0001	105.42	−9.25	0.8158	0.0585	−1.25	0.1788	2.23	0.265
C-Lipid	0.2519	1.56	−1.13	0.8158	0.0585	−1.25	<0.0001	1012.98	5.65
AB	0.85	0.0385	−0.25	0.7423	0.1171	2.5	0.5257	0.4459	−0.1675
AC	0.7064	0.154	−0.5	0.1309	2.93	12.5	0.0757	4.34	−0.5225
BC	0.85	0.0385	0.25	1	0	0	0.0164	9.86	0.7875
A^2	0.54	0.415	0.8	0.0001	55.38	53	0.0095	12.53	−0.8653
B^2	0.4693	0.5853	−0.95	0.946	0.0049	0.5	0.0753	4.36	−0.5103
C^2	0.1198	3.14	−2.2	0.946	0.0049	0.5	0.1218	3.1	−0.4302
R^2 Value	0.9411			0.9945			0.9934		

time, 1090 cps of viscosity, and 86 % of in vitro drug release study, which is almost similar to the FRF 8 batch and predicted value. The desirability plot and overlay plot are depicted in Fig. 11(a and b).

4.12. Drug release kinetics

The mechanism of drug release involved in optimized FRF was analyzed through mathematical models like Zero order, First order, Higuchi, and Korsemeyer Peppas equation using the release data of acyclovir. The R2 value for various tested models is given in Table 6. Higuchi and zero order kinetics give a similar R2 value of 0.952 and 0.994, and the n value is 0.552 and 0.545 respectively. The acyclovir FRF follows a non-fickian release mechanism. This indicates the best-fit model was found to be the zero-order kinetics and had an R2 value of 0.994, with anomalous non-Fickian transport diffusion as the release mechanism (release exponent, n = 0.545) for FRF formulation. All the kinetic models of optimized batch were depicted in Fig. 12.

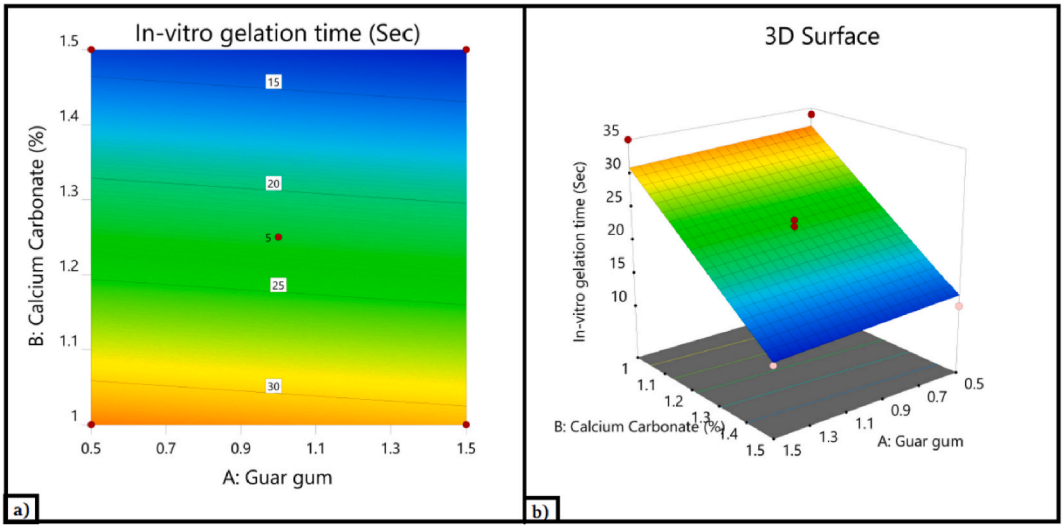


Fig. 8. Contour plot (a) and Three dimensional surface plot (b) depicting the effect of Guar gum and calcium carbonate on *in-vitro* gelation time (sec).

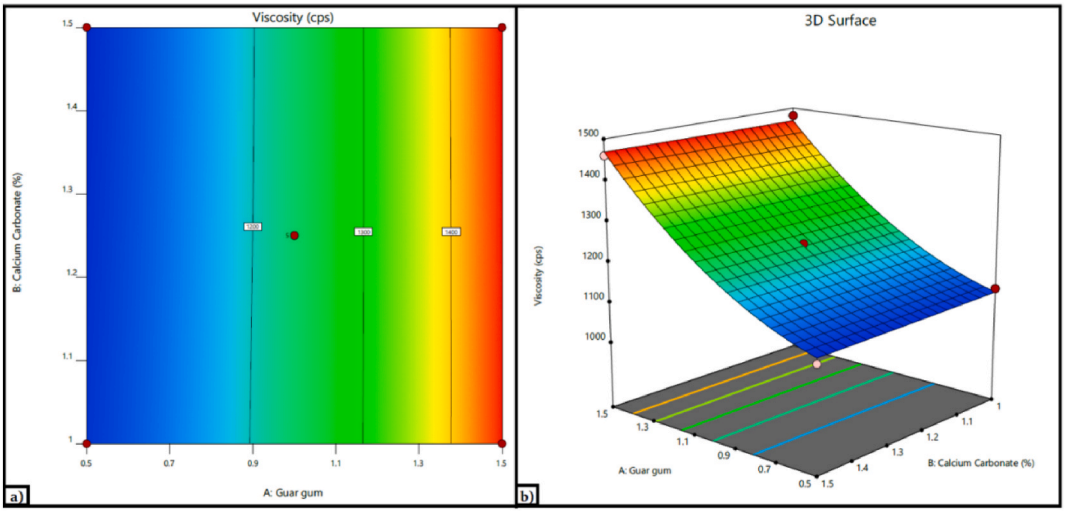


Fig. 9. Contour plot (a) and Three dimensional surface plot (b) depicting the effect of Guar gum and calcium carbonate on Viscosity (cps).

4.13. In vitro permeability study

The results of the in vitro permeability study of an optimized batch of Acyclovir FRF are depicted in Fig. 13. The cumulated amount of drug permeated for the duration of 12 h. The flux for the optimized batch was found to be 0.6914 μg/cm2/h, and the permeability coefficient for the optimized batch is 0.03457cm2/h, as depicted in Table 7. The flux and permeability coefficients for the optimized acyclovir FRF batch have a higher value, indicating the drug is highly permeated because of guar gum acting as an emulsifier and carbopol as a permeation enhancer present in the formulation, enhancing the permeability.

5. Conclusion

The guar gum and GMS-based FRF system were optimized by BBD. Three variables and three responses were selected to meet the requirements of FRF for achieving the bioavailability of acyclovir. This conclusively demonstrates that the optimal amount of FRF containing guar gum (0.5), GMS (1.25), and calcium carbonate (1.5) has excellent floating and gelation times and also retards the drug release of acyclovir. Seventeen batches were developed with three different variables, and their interactions between responses were analyzed through the polynomial equation, contour, and 3D plot. The optimized batch of acyclovir FRF has an optimal effect on GRT, which is analyzed through rheological studies. The in vitro permeability study confirms that acyclovir FRF has improved the

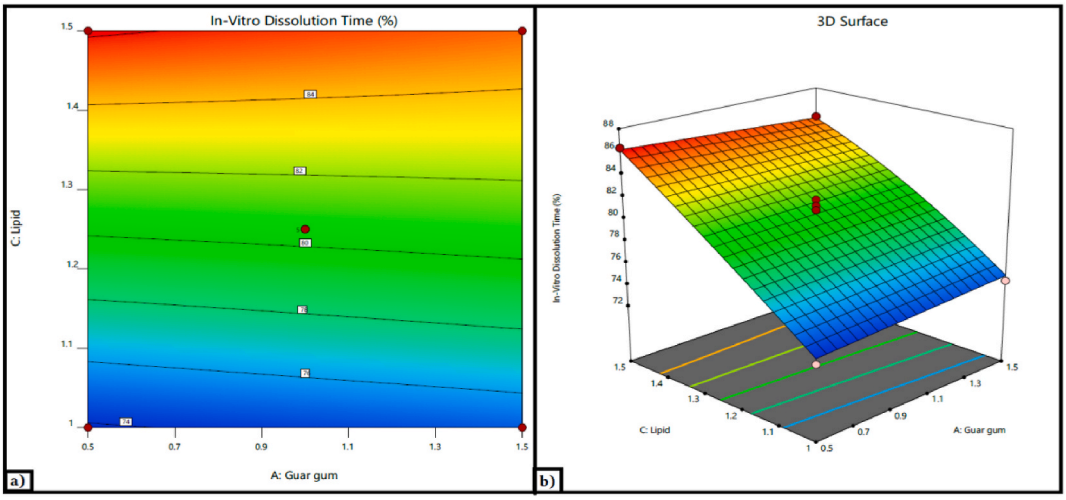


Fig. 10. Contour plot (a) and Three dimensional surface plot (b) depicting the effect of Guar gum and Lipid on *in-vitro* drug release study (%).

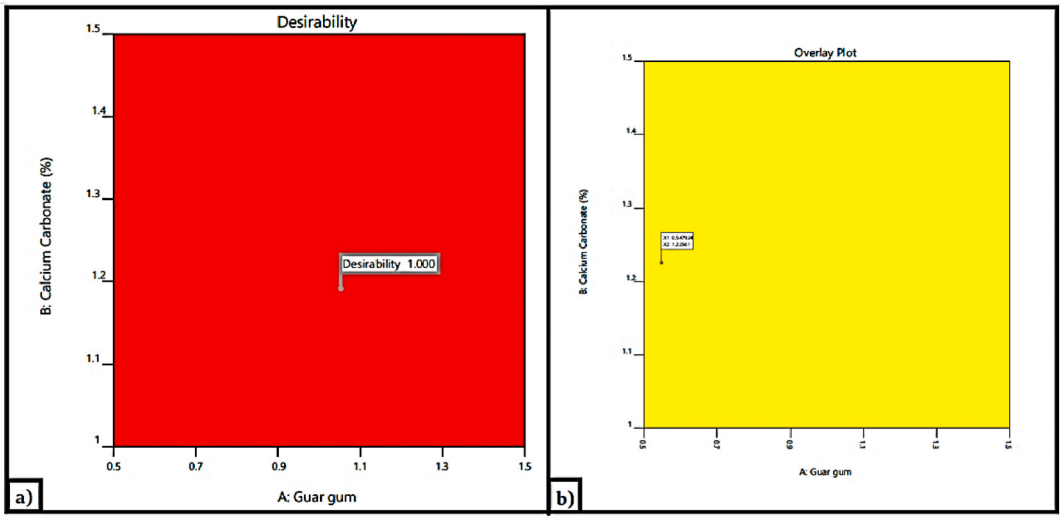


Fig. 11. (a) Desirability plot for obtained results. (b) Overlay plot for the obtained results.

Table 6
R^2 value of Kinetic models.

Batch	Zero order	First Order	Korsmeyer Peppas	Higuchi	Best fit model
R^2 Value of Optimized batch	0.994	0.8526	0.432	0.9526	Zero order kinetic and Non – fickian transport
n Value	0.545	−0.0799	0.0434	0.552	
K value	0.614	0.1768	0.5457	0.643	

absorption of acyclovir. The results demonstrate that the guar gum-GMS-based FRF system significantly enhances the absorption of acyclovir by enhancing GRT and prolonging the drug release in the stomach.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

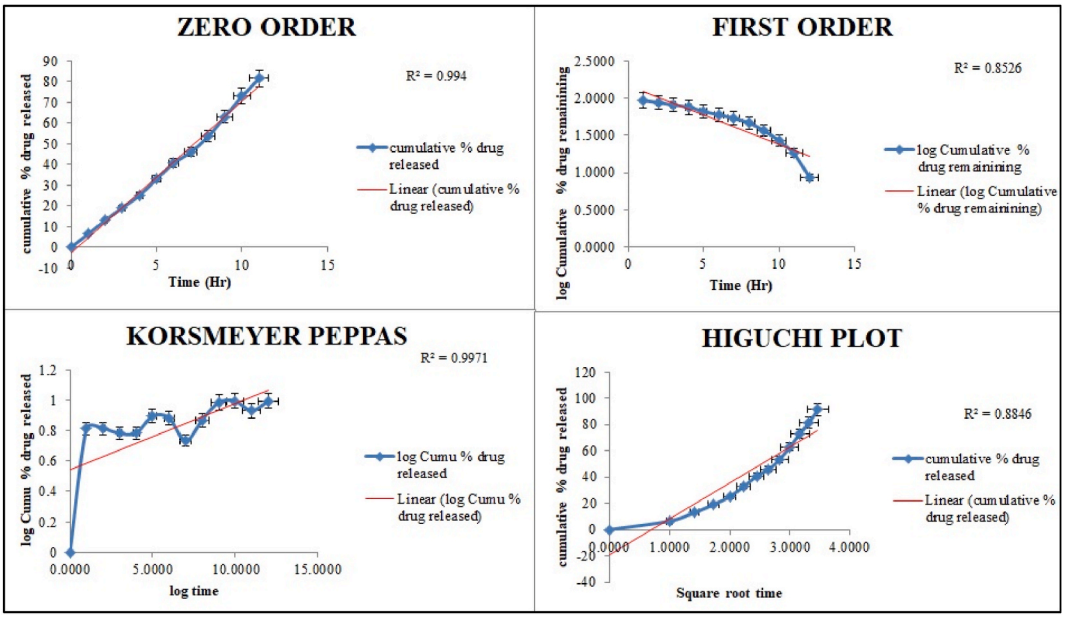


Fig. 12. Depicting the mathematical model for optimized batch.

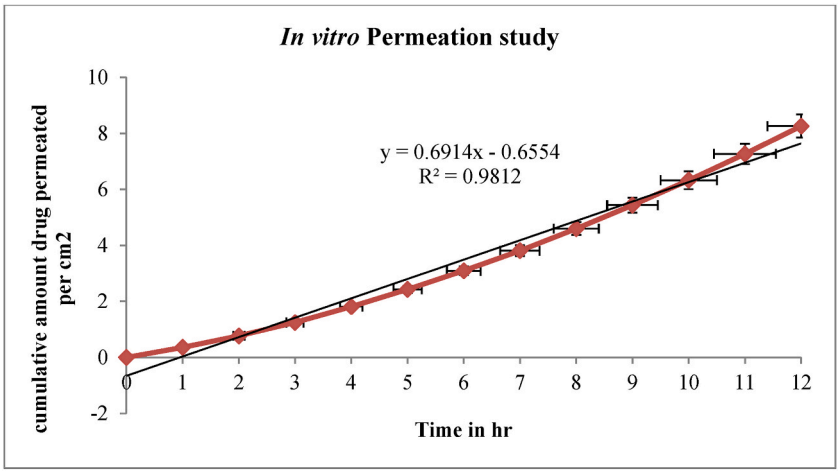


Fig. 13. *In vitro* permeability study for Optimized batch.

Table 7
Cumulative amount of drug permeated in Optimized batch.

Formulation	Flux (Jss, μg/cm^2/h)	Permeability coefficient (Kp, cm^2/h)
Optimized batch	0.6914	0.03457

Ethics approval

This is an observational study (*In-vitro* study). The enhanced gastric residence time of acyclovir by floating raft formulation using box-behnken design of Research Ethics Committee has confirmed that no ethical approval is required.

Data availability statement

The raw/processed data required to reproduce the above findings cannot be shared at this time as the data also forms part of an ongoing study and on request the data will be made available for readers.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Rajalakshmi Munusamy: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Sangeetha Shanmugasundharam:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors would like to thank Department of Pharmaceutics, SRM College of Pharmacy, SRMIST, Kattankulathur for providing facilities and guidance to carry out the research.

References

[1] U. Bhosale, D.V. Kusum, N. Jain, Formulation and optimization of mucoadhesive nanodrug delivery system of acyclovir, J. Young Pharm. 3 (2011) 275–283, https://doi.org/10.4103/0975-1483.90236.
[2] H. Hassan, R. Omenes o bello, Khadijah Adam, Alias siti, E. Meor Mohd Redzuan Meor Mohd Affandi, Acyclovir-loaded solid lipid nanoparticles: optimization, characterization and evaluation of its pharmacokinetic profile - PMC, Nanomaterials 10 (2020) 1785, https://doi.org/10.3390/nano10091785.
[3] S. Shin, T.H. Kim, S.W. Jeong, S.E. Chung, D.Y. Lee, D.H. Kim, et al., Development of a gastroretentive delivery system for acyclovir by 3D printing technology and its in vivo pharmacokinetic evaluation in Beagle dogs, PLoS One 14 (2019) 1–17, https://doi.org/10.1371/journal.pone.0216875.
[4] A.B. Nair, M. Attimarad, B.E. Al-Dhubiab, J. Wadhwa, S. Harsha, M. Ahmed, Enhanced oral bioavailability of acyclovir by inclusion complex using hydroxypropyl-β-cyclodextrin, Drug Deliv. 21 (2014) 540–547, https://doi.org/10.3109/10717544.2013.853213.
[5] R. Bahri-Najafi, A. Mostafavi, N. Tavakoli, S. Taymouri, M.-M. Shahraki, Preparation and in vitro-in vivo evaluation of acyclovir floating tablets, Res Pharm Sci 12 (2017) 128–136, https://doi.org/10.4103/1735-5362.202451.
[6] M. Ibrahim, Y.W. Naguib, H.A. Sarhan, H. Abdelkader, Gastro-retentive oral drug delivery systems: a promising approach for narrow absorption window drugs, J Adv Biomed & Pharm Sci J Adv Biomed & Pharm Sci 2 (2019) 98–111.
[7] D.S. Kumar, D.E. Tony, A.P. Kumar, K.A. Kumar, D.B.S. Rao, R. Nadendla, A REVIEW ON : ABELMOSCHUS ESCULENTUS (OKRA) 3 (2013) 129–132.
[8] R. Patel, G. Vaishnav, Raft forming system - gastroretentive drug delivery system, International Journal of Advanced Engineering, Management and Science 6 (2020) 515–519, https://doi.org/10.22161/IJAEMS.612.5.
[9] M. Kumar, D. Kaushik, An overview on various approaches and recent patents on gastroretentive drug delivery systems, Recent Pat. Drug Deliv. Formulation 12 (2018) 84–92.
[10] V.D. Prajapati, G.K. Jani, T.A. Khutliwala, B.S. Zala, Raft forming system—an upcoming approach of gastroretentive drug delivery system, J. Contr. Release 168 (2013) 151–165, https://doi.org/10.1016/J.JCONREL.2013.02.028.
[11] S. Manna, K. Jayasri, K.R. Annapurna, L.K. Kanthal, Alginate based gastro-retentive raft forming tablets for enhanced bioavailability of tinidazole, Int. J. Appl. Pharm. 9 (2017) 16–21, https://doi.org/10.22159/ijap.2017v9i1.15757.
[12] V. Joshi, R. Awasthi, Floating in-situ raft forming liquid gastroretentive drug delivery system containing poorly water-soluble drug, Bulletin of Pharmaceutical Sciences Assiut 44 (2021) 301–312, https://doi.org/10.21608/BFSA.2021.207147.
[13] M. Hanif, G. Abbas, S. Shah, M. Zaman, A. Rasul, A. Majeed, et al., Raft-forming system for pantoprazole and domperidone delivery: in vitro and in vivo study, Bioinspired, Biomimetic Nanobiomaterials 9 (2020) 137–146, https://doi.org/10.1680/jbibn.19.00031.
[14] A. Mortensen, F. Aguilar, R. Crebelli, A. Di Domenico, M.J. Frutos, P. Galtier, et al., Re-evaluation of guar gum (E 412) as a food additive, EFSA J. 15 (2017) e04669, https://doi.org/10.2903/j.efsa.2017.4669.
[15] M. Maboos, R.I. Yousuf, M.H. Shoaib, I. Nasiri, T. Hussain, H.F. Ahmed, et al., Effect of lipid and cellulose based matrix former on the release of highly soluble drug from extruded/spheronized, sintered and compacted pellets, Lipids Health Dis. 17 (2018) 136, https://doi.org/10.1186/s12944-018-0783-8.
[16] M. Moganti, H.N. Shivakumar, Oral raft forming in situ gelling system for site specific delivery of calcium, J. Drug Deliv. Sci. Technol. 61 (2021) 102113, https://doi.org/10.1016/j.jddst.2020.102113.
[17] R. Omar, M.S. Aboraia, E.A. Oraby, R. Gubner, A.E. Rizk, The effect of sodium citrate as a complex agent on the corrosion properties of the electroless Ni-P coating, Mater. Res. Express 5 (2018) 126511, https://doi.org/10.1088/2053-1591/aae0b9.
[18] J. Hao, X. Fang, Y. Zhou, J. Wang, F. Guo, F. Li, et al., Development and optimization of solid lipid nanoparticle formulation for ophthalmic delivery of chloramphenicol using a Box-Behnken design, Int. J. Nanomed. 6 (2011) 683–692, https://doi.org/10.2147/ijn.s17386.
[19] M.K.M. Darwish, A.S.M. Abu El-Enin, K.H.A. Mohammed, Formulation, optimization, and evaluation of raft-forming formulations containing Nizatidine, Drug Dev. Ind. Pharm. 45 (2019) 651–663, https://doi.org/10.1080/03639045.2019.1569033.
[20] N.A.H. Abou Youssef, A.A. Kassem, M.A.E. El-Massik, N.A. Boraie, Development of gastroretentive metronidazole floating raft system for targeting Helicobacter pylori, Int. J. Pharm. 486 (2015) 297–305, https://doi.org/10.1016/j.ijpharm.2015.04.004.
[21] S. Al-Lami M, Formulation and evaluation of sustained and raft forming antacid tablet, Iraqi Journal of Pharmaceutical Sciences (P-ISSN: 1683 - 3597 , E-ISSN : 2521 - 3512) 26 (2017) 26–31, https://doi.org/10.31351/vol26iss1pp26-31.
[22] U. Hani, M. Rahamathulla, R.A.M. Osmani, Y. Begum, S. Wahab, M. Ghazwani, et al., Development and characterization of oral raft forming in situ gelling system of neratinib anticancer drug using 32 factorial design, Polymers 14 (2022), https://doi.org/10.3390/polym14132520.
[23] V.S. Meka, S.R. Nali, A.S. Songa, J.R. Battu, V.R.M. Kolapalli, Statistical optimization of a novel excipient (CMEC) based gastro retentive floating tablets of propranolol HCl and it's in vivo buoyancy characterization in healthy human volunteers, DARU J Pharm Sci 20 (2012) 21, https://doi.org/10.1186/2008-2231-20-21.
[24] M.M. Al-Tabakha, S.A. Khan, A. Ashames, H. Ullah, K. Ullah, G. Murtaza, et al., Synthesis, characterization and safety evaluation of sericin-based hydrogels for controlled delivery of acyclovir, Pharmaceuticals 14 (2021) 234, https://doi.org/10.3390/ph14030234.
[25] Haniza Hassan, Siti khadijah adam, ekram alias second, meor mohd redzuan meor mohd affandi third, Ahmad Fuad Shamsuddin fourth, Rusliza Basir. Central composite design for formulation and optimization of solid lipid nanoparticles to enhance oral bioavailability of acyclovir, Molecules 26 (2021) 5432, https://doi.org/10.3390/molecules26185432.

[26] S.M. Abouelatta, A.A. Aboelwafa, O.N. El-Gazayerly, Gastroretentive raft liquid delivery system as a new approach to release extension for carrier-mediated drug, Drug Deliv. 25 (2018) 1161–1174, https://doi.org/10.1080/10717544.2018.1474969/SUPPL_FILE/IDRD_A_1474969_SM0137.MP4.
[27] G. Gananarajan, R. Pal, Raft forming gastro retentive drug delivery system-a novel, Approach 10 (2021) 650–659, https://doi.org/10.20959/wjpps20218-19519.
[28] D.N. Bhavsar, Advances in grdds: raft forming system a review, J. Drug Deliv. Therapeut. 2 (2012) 123–128, https://doi.org/10.22270/jddt.v2i5.228.
[29] H. Soni, V.A. Patel, Preparation and evaluation of raft forming, Chewable Tablets of Ranitidine Hydrochloride 5 (2016) 290–296.
[30] S.S R, H.A S. IN-SITU RAFT FORMING SYSTEM: A REVIEW | INTERNATIONAL JOURNAL OF PHARMACOGNOSY. International Journal of Pharmacognosy n.d.; 5:337–349. https://doi.org/10.13040/IJPSR.0975-8232.IJP.5(6).337-49..
[31] S. Dash, P.N. Murthy, L. Nath, P. Chowdhury, Kinetic modeling on drug release from controlled drug delivery systems, Acta Poloniae Pharmaceutica - Drug Research 67 (2010) 217–223.
[32] H Salamanca C, A. Barrera-ocampo, Franz Diffusion Cell Approach for Pre-formulation Characterisation of Ketoprofen Semi-solid Dosage Forms, vols. 1–10, 2018, https://doi.org/10.3390/pharmaceutics10030148.