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Research article

DEVELOPMENT AND CHARACTERIZATION OF TOPICAL OLEO-GEL SYSTEM OF CEFUROXIME AXETIL

Neha Javia, Het Solanki, Grishma Patel, Lata Panchal*

Department of Pharmaceutics,, K.B. Institute of Pharmaceutical Education and Research, A constituent college of Kadi Sarva Vishwavidyalaya, Sector-23, Gandhinagar, Gujarat, India

Abstract

Oleogel is a semisolid matrix that may change shape when heated or cooled. It is made of an apolar substance that comes together on its own. Within a three-dimensional network made up of several oleogelators and co-oleogelators that interact chemically or physically, the phase is contained. The simplicity of manufacture and long-term stability of oleogel-based products make them unique. Using Span 65 as the oleogelator and Tween 80 as the co-oleogelator, this study sought to create an oleogel made from sunflower oil that included the antimicrobial drug Cefuroxime axetil for use in wound healing. By varying the amounts of Span 65 and Tween 80 in each formulation, we created a number of oleogel formulations. It was shown that 16% was the critical gelator concentration (CGC) of Span 65 in sunflower oil. Cefuroxime axetil was included into the oleogel for its antibacterial properties. The prepared oleogel was assessed for organoleptic characteristics, spreadability, oil binding capacity (%OBC), gel-sol transition temperature (Tg), viscosity, and release of drugs in vitro research. The optimization of several oleogels was conducted using a 32 complete factorial design, examining the influence of Span 65, Tween 80, and oil quantity on % OBC, viscosity, gel-sol transition temperature, and release of drugs in vitro at 1 hour and 5 hours. The optimal oleogel was determined to consist of a moderate quantity of Span65, an elevated quantity of Tween80, and a fixed volume of oil (25 ml). The improved oleogel underwent antibacterial and expedited stability studies.

Keywords: Cefuroxime axetil, Wound healing, Oleogel, Oleogelator, Co-oleogelator, Antibacterial, Sunflower oil

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*Corresponding Author

Introduction

Wound infection represents a significant issue in contemporary medical science, particularly in developing nations, where it predominantly arises from unsanitary conditions and limited access to healthcare resources. A wound constitutes a physical, mechanical, or thermal injury to the skin, resulting in the disruption of epithelial continuity. Wound care management encompasses the repair the repair of both anatomical and functional soundness while minimizing the risk of infection and scarring in the affected tissue. Wound healing is a complicated biological process that happens over a certain amount of time and involves the coordinated activity of different kinds of cells, cytokines, and growth hormones. There are four stages that partly overlap in the process: hemostasis, inflammation, proliferation, and remodeling. All these steps must interact precisely at the cellular and molecular levels to promote properly healed tissue. Bacterial infection or inadequate vascularization can disrupt this sequence of events, leading to delayed healing and potentially resulting in chronic wound conditions. Consequently, the development of advanced topical formulations that enhance wound healing and deliver localized antibacterial effects is of significant importance. [1] Oleogels have garnered significant attention as novel drug delivery systems due to their potential applications in topical and transdermal administration. Oleogels are semi-solid systems formed by the immobilization of liquid oil within a three-dimensional network created by gelators, including waxes, fatty acids, or biopolymers. Their unique structure provides several benefits, including enhanced drug solubilization, thermodynamic stability, increased skin permeation, and controlled release. Oleogels, characterized by their lipid-rich composition, are particularly effective for delivering lipophilic drugs or those exhibiting poor water solubility. Additionally, they demonstrate superior spreadability, bioadhesive characteristics, and compatibility with various pharmaceutical and cosmetic active ingredients. The performance of an oleogel is significantly influenced by the type of oil and gelator utilized. Oils exhibiting higher viscosity, lower polarity, and increased unsaturation typically form stronger and more stable gels. The type of oleogelator, whether low molecular weight (e.g., waxes, fatty acids) or high molecular weight (e.g., proteins, polysaccharides), influences the mechanical and rheological properties as well as the release behavior of the formulation. These properties can be systematically designed to produce targeted therapeutic outcomes, establishing oleogels as a flexible medium for topical drug administration. Cefuroxime axetil is a second-generation cephalosporin antibiotic that fights a lot of different types of bacteria, both Gram-positive and Gram-negative. It is typically indicated for infections of the respiratory, urinary, skin, and soft tissues. [2] Cefuroxime axetil is a white crystalline powder with the molecular formula $C_{20}H_{22}N_4O_{10}S$ and a molecular weight of 510.47 g/mol. The compound exhibits moderate oral bioavailability of approximately 67.9%, a brief biological half-life of around three hours, and plasma protein binding of roughly 50%. This drug is classified as BCS Class II, characterized by low aqueous solubility and high permeability, leading to variable absorption and reduced oral bioavailability. The topical administration of cefuroxime axetil formulated with an oleogel base may address several of these limitations. [3]The lipid matrix of the oleogel enhances drug solubility and facilitates skin diffusion, promoting localized antibacterial action while circumventing first-pass metabolism and gastrointestinal degradation. This approach facilitates sustained release of the active drug at the infection site, enhancing therapeutic efficacy and patient compliance. This study focuses on the formulation and evaluation of a Cefuroxime axetil-based oleogel designed for topical use in managing wound infections. This paper aims to optimize the oleogel system's composition to achieve desirable physicochemical properties, drug release behavior, and antimicrobial activity, thereby establishing a stable and effective alternative to conventional oral therapy.

Materials and methods

Cefuroxime axetil was obtained as gift samples from Redson Pharmaceuticals Pvt Ltd. Additionally, Polyethylene glycol 400 (PEG) and hydroxypropyl methylcellulose E5 (HPMC) were purchased from Oxford Lab Fine Chem LLP. Carbopol 934P was procured from Biochem Pvt Ltd.

Methods

2.1. Analytical Method Development

Solution of Cefuroxime axetil 10µg/ml was prepared in methanol. The solution was observed for maxima between 200-400 nm using spectrophotometer (shimadzu UV-1800, Japan). An accurately weighed quantity (25 mg) of Cefuroxime Axetile was put into a volumetric flask (25 ml). It was dissolved in methanol to reach the ultimate level of concentration of the stock solution as 1000 µg/ml of cefuroxime axetil. 5 ml of the stock solution was further diluted to 50 ml with the respective solvent system to obtain working standard solution (100µg/ml). similarly same procedure was followed for PBS pH 5.5 and 20% methanolic PBS 5.5. [4]

2.2. Drug Authentication

2.2.1. Melting Point Determination

We used the capillary technique and a melting point equipment to find out what temperature the medication melted at. A little amount of the medicine was put into a capillary tube that had been sealed at one end. The capillary was then put in a melting point machine, and the temperature at which the material began to melt was recorded.

2.2.2. FTIR of Cefuroxime axetil

An FTIR spectrophotometer (FTIR-8400S, Shimadzu, Kyoto, Japan) was employed to obtain the Fourier

transform infrared (FTIR) spectra of Cefuroxime axetil and Cefuroxime axetil infused Oleogel. Samples were combined with potassium bromide (FT-IR grade) and subsequently subjected to a hydraulic press to form disks. Subsequently, we conducted scans from 4000 to 500 cm−1. [5]

2.3. Formulation and characterization of oleogel

2.3.1. Screening of Oleogel Components

Five milliliters of each of the following oils—almond, anise, coconut, olive, oleic acid, sunflower, and tea—contained an excessive concentration of Cefuroxime axetil molecules. The drug's solubility in various oils was evaluated by placing the samples into individual 10-mL stoppered vials and subjecting them to vigorous mixing with a vortex mixer. The combined vials were maintained at 25±1.0°C for 72 hours in an isothermal shaker to achieve equilibrium. 6. Subsequent to the equilibration of the samples, they were removed from the shaker and subjected to centrifugation at 3,000 rpm for 15 minutes. A 0.22 μm membrane filter was used to collect and purify the supernatant. The concentration of cefuroxime axetil in the oils was quantified using UV spectroscopy at 271 nm. The minimum gelator quantity required to maintain the stability of an apolar liquid inside an oleogel is referred to as the Critical Gelator Concentration (CGC). The critical gelator concentration (CGC) was determined by increasing the concentration of Span-65 (oleogelator) in sunflower oil (solvent) from 10% to 20% (w/w) in a beaker at 55°C. The beakers were inverted to determine the critical gelation concentration (CGC) of the oleogel. During the examination, the oleogel exhibited no fluidity.

2.3.2 Preparation Of Oleogel Formulation

Precisely quantified Span 65 (oleogelator) and Tween 80 (co-oleogelator) were combined with a specified quantity of sunflower oil and maintained at 60 °C while being magnetically stirred at 500 rpm for a duration of 15 to 20 minutes. After that, the solution was brought down to a normal room temperature to help it gel. The solution not flowing under gravity when turned upside down showed that gelation had worked. We found the critical gelator percentage (CGC) by changing the quantity of Span-65. The CGC represents the minimum quantity of gelator required to prevent the movement of the apolar liquid. Utilize an analytical balance to obtain the precise quantity of sunflower oil required. Pour the oil into a clean, dry beaker. Position the beaker filled with sunflower oil on a magnetic stirrer. [7] Commence churning the oil at a moderate velocity. Incrementally introduce 250 mg of the pharmaceutical into the beaker. Persist in stirring until the pharmaceutical compound is entirely dissolved in the sunflower oil. Verify that the medication is completely solubilized to get a homogeneous solution. After the medication is completely dissolved in the sunflower oil, include Tween80 (a polymer) into the mixture while maintaining agitation. Once the Tween is completely blended, add Span65 (oleogelator) into the solution. Elevate the temperature of the beaker holding the mixture over 60°C while maintaining agitation. Regulate the temperature and agitation until a homogeneous solution is obtained. Detach the beaker from the heat source. Permit the solution to cool somewhat. Allow the beaker to sit at room temperature overnight to facilitate gel formation.

2.3.3. Characterization of Oleogel:

2.3.3.1 Organoleptic evaluation:

The newly synthesized oleogels underwent organoleptic assessment by examining their gel formation, color, odor, appearance, and texture.

2.3.3.2 Oil binding capacity (%OBC):

A altered version of Yılmaz and Ögütcü's approach was employed to measure this attribute. We used a scale to weigh the emptied Eppendorf tubes and put the right labels on them. After that, 1 g of every oleogel from every batch was put through an Eppendorf tube, weighed again, and written down. The Eppendorf tubes had been stored in the fridge for an hour at 4 °C. [8] After being put in the fridge, the eppendorf tubes were weighed again and then spun in a centrifuge to stay 15 minutes at 10,000 rpm.

Following centrifugation, the supernatant (oil) was removed from the Eppendorf tubes, which were subsequently reweighed. We repeated this process three times, after which we applied the formula to obtain the OBC.

$$\% \text{ Released oil} = (b - a) - (c - a)/ (b - a) \times 100$$

$$\% \text{ OBC} = 100 - \text{Released oil}$$

Where:

a = weight of empty eppendorf tube

b = weight of eppendorf tube after refrigeration

c = weight of eppendorf tube after centrifugation and removing supernant

2.3.3.3 Thermal analysis:

We employed the falling ball method to figure out the oleogels' gel to sol temperatures of transition (Tg). The study employed a ball made of stainless steel that was 1/8 inch in diameter and weighed 130 milligrams. [9] The melting point equipment's the sample container had exactly 1 g of oleogel in it. An SS ball was placed over the sample, and the temperature was maintained at 25 °C. The samples had been heated at an average speed of 1°C/min, along with the temperature that occurred when the ball started to move was noted as the oleogel's Tg.

2.3.3.4 Spreadability studies:

We used the standard method to test how well the oleogels dispersed. A predetermined mass of examples (0.5 g) was placed between two plates of glass of equal weight and surface area. Before the weight was put on, the first spreading diameter was measured. After that, 10 g, 20 g, 50 g, and 100 g weights were put on the top glass plate. It took 60 seconds to record the final diameter. We used the following equation to figure out the spreading ability of the compounds as a percentage.

The percentage of Spreadability = (M × L) / T, where M is the mass attached to the top plate, L is the overall length of the plate, and T is the length of time it takes to separate the two plates.

2.3.3.5 Viscosity measurement:
The manufactured formulations were analysed for viscosity using a Brookfield viscometer with Spindle number 62, going at 100 rpm while maintaining room temperature.

2.3.3.6 Studies on drug release in vitro:
A modified Franz diffusion cell used to investigate drug release. The diffusion cell is composed of a donor part and a receptor part. A cellulose acetate membrane divides the two parts. Following measurement, the donor chamber was filled with precisely one gram of oleogel. The receptor media was composed of methanol in an 8:2 ratio and phosphate buffer with a pH of 5.5. Throughout, the receptor fluid was spun at 50 rpm and maintained at 37 ± 0.5 °C. For the first hour, the receptor's total volume changed every 15 minutes, and for the next five, it changed every hour. A 5 ml sample with a λmax of 276 nm was evaluated for spectrophotometric characteristics using a double-beam UV-visible spectrophotometer.

2.3.3.7 Application of 3^2 full factorial design in optimization of oleogel:
This research used a 3^2 complete factorial design to improve the oleogel formulation. Unlike the conventional trial and error approach, which necessitates several batches and demands significant work and time, factorial design is an effective technique for producing complicated formulations with a minimal number of batches in the shortest timeframe. It also denotes the comparative importance of selection variables and their interactions. This design assessed two independent variables, All of the three different levels, with trials in the lab conducted for all nine potential arrangements. [10] The elevated, moderate, and diminished values of each factor are encoded as 1, 0, and -1, respectively. The values of the formulation factors were selected based on the findings obtained from preliminary research. Consequently, these factors were used to determine the optimal recipe for producing oleogel. Alongside factors and levels, dependent variables were also chosen for the assessment of the factorial design batches. The dependent variables were Oil Binding Capacity (%OBC) (Y1), Gel-Sol Transition Temperature (Tg) (Y2), Viscosity (Y3), and In Vitro Drug Release (%CPR at 6 hours).

2.3.3.8 In vitro Antifungal study:
Through in vitro antifungal agents investigation was conducted utilizing the Using the "cup-plate method," the Sabouraud dextrose agar diffusion disk test was done on a Petri dish that had already been cleaned. Oleogel samples, both drug-loaded and drug-free, were inserted into 8mm cups and then positioned in wells of a Sabouraud dextrose plate that had been inoculated with the test organism Candida albicans. Following a 2-hour diffusion of the solution, the plate was incubated at 27 °C for 24 hours.[11] The area surrounding the cup where bacteria couldn't grow was compared to the blank oleogel. The outcomes of triple measurements and their averages were recorded.

2.3.3.9 Accelerated stability study:
Accelerated stability investigations were conducted using the thermo-cycling or freeze-thaw cycling approach. Freshly manufactured oleogel samples underwent several freezing-thawing cycles, alternating During 15 minutes each at temperatures between -20°C and 60°C, for a total duration of 4 hours (i.e., 16 cycles). Subsequently, the batches were examined for phase separation or any other instability characteristics. A sample is considered stable if it endures a minimum of 5 rounds of thermo-cycling. [12]

Results and discussion

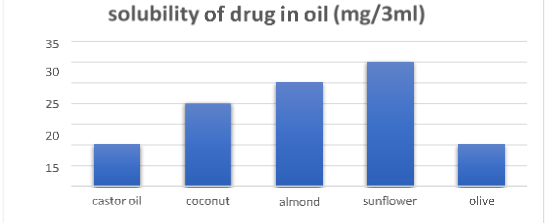
3.1. The characteristic peak of Cefuroxime axetil was observed at 276 nm. UV spectra of cefuroxime axetil in Methanol is Cefuroxime axetil followed beer lambert's law in concentration range of 2.5 – 25 µg/ml. High value of correlation coefficient indicate that is a linear between concentration and absorbance. Cefuroxime axetil followed Beer Lambert's law in concentration range of 2.5- 25 µg/ml. High value of correlation coefficient (0.9934) indicate that is a linear relation between concentration and absorbance. [13]

3.2 Authentication of the drug
3.2.1. Melting point
It was found that Cefuroxime axetil melts at between 171.5°C and 173°C, which aligns well with the literature data.

3.2.2. FTIR Analysis
Illustrates the four peaks located at 812.03, 1585.49, 1739.79, and 1286.52 cm^{-1}. The FTIR spectra of the drug-excipients physical combination exhibit similar absorption peaks to those of the drug alone, indicating the lack of any interaction between Cefuroxime and the excipients. [14]

3.3 Formulation and characterization of Oleogel
3.4 3.3.1 Screening of oleogel components by saturation solubility study.

Figure 1: Solubility of drug in oil

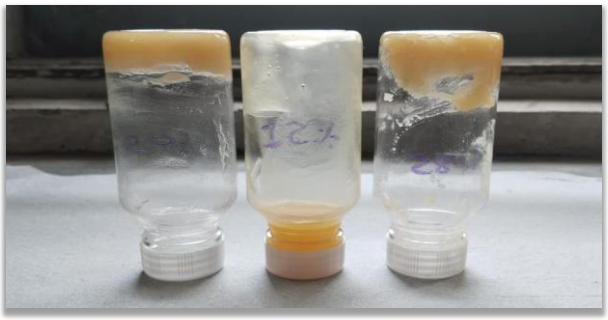


The table indicates that oils such as castor oil, coconut oil, almond oil, sunflower oil, and olive oil exhibit solubility of cefuroxime axetil, with sunflower oil demonstrating the maximum solubility of this compound. Sunflower oil was used as a solvent in the manufacture of oleo gel. Moreover, sunflower oil is rich in vitamin E, which facilitates skin regeneration and offers protection against oxidative damage, so aiding tissue repair.

3.3.2 CGC (critical gelation concentration) determination

The CGC of the oleogelator (span65) was found to be 16% (w/w) in sunflower oil. Below 16%(w/w) concentration of span 65, all the sample was started flowing when beaker was inverted. [15]

Figure 2: CGC of various batches



3.3.3 Organoleptic evaluation

The organoleptic assessment findings of all batches in indicate that all manufactured oleogel samples had a yellowish hue, attributable to the intrinsic color of Span 65. [16] They emitted a faint odor and had a smooth, oily texture. With the augmentation of oleogelator content, the viscosity of the oleogel intensified. The study noted that oleogels with a greater mix of oleogelator and co-oleogelator achieved the gel structure more rapidly.

.3.4 Spreadability of batches OG1-OG9

As shown in figure 4, all batches OG1-OG9 have optimum spreadability in range of 11 to 60 (gcm/sec).

3.3.5 Determination of %OBC (oil binding capacity)

Here show that batch second, batch four and batch seven has a maximum oil binding capacity.[17]

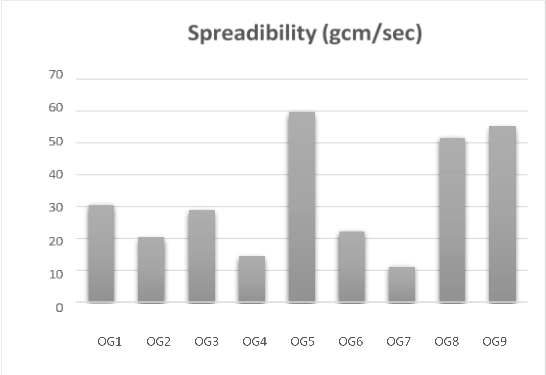
We conclude that as the amount of span increased oil binding capacity was increased.

3.3.6 Viscosity of batches OG1-OG9

As shown in figure 6, lowest viscosity (132 cps) was observed for run number 5 and 9 the highest viscosity of the oleo gel found was 1375 cps. we conclude that as the amount of span increased viscosity was increased.

Figure 3: OG1 toOG9 all batches

Figure 4: Spreadability of various batches



3.3.7 %Cumulative In vitro release of drugs of batch

Studies on drug release in vitro were conducted.out for all the formulation and from the results obtained as shown in figure 7 states that the oleo gel could permeate easily through the membrane. Out of all the formulation F6 showed 18 whichis highest % of drug release in 1 hour and 75 which is highest % of drug release in 5 hours.[18]

3.3.8 pH of batches OG1-OG9

The pH of semisolid formulations influences the solubility and stability of the incorporated drug, as well as its potential for skin irritation. [19] Oleogel formulations must maintain pH values within the physiologically recognized range of 4.5 to 6.5 to ensure safety and non-irritation. The pH levels of all the geared up oleogel formulations were included in the specified range. [20]

Figure 5: %OBC of all batches

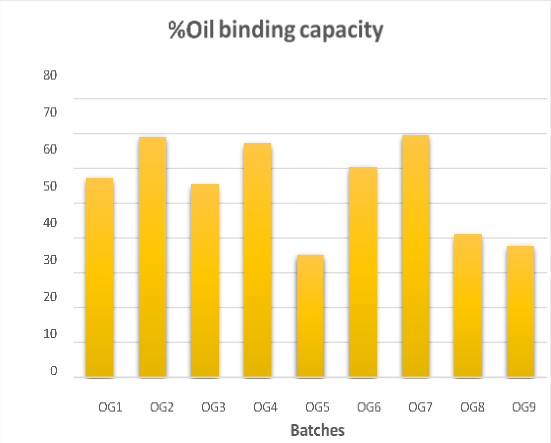
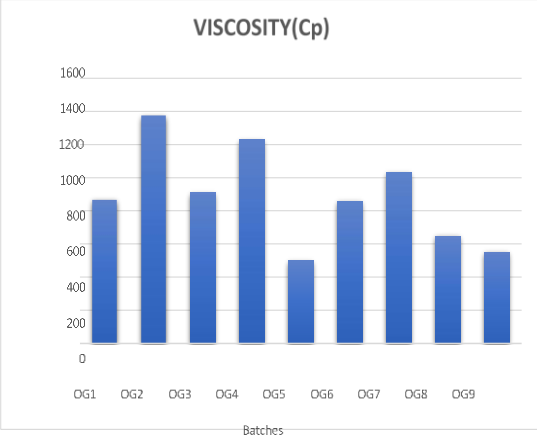


Figure 6: Viscosity of all batches



3.3.9 Statistical analysis of data and validation of factorial design

All nine batches were prepared with parent formula. The result of oil binding capacity, viscosity and drug release at 1 hour and 5 hours were noted in table 2. [21]

3.3.9.1 Effect of independent variable on % OBC Y1: Oil absorption capability. The findings of the multiple linear regression analysis indicated that coefficient A for X1 is -0.8650, whereas coefficient B for X2 is 15.30. X1 negatively impacted oil binding ability, but X2 positively influenced it. Therefore, we deduce that an increase in span correlates with an enhancement in oil binding ability. X2 had a greater influence on oil binding capability than the X1 variable. [22]

The modified equation for response Y1 is shown below:

$$\%OBC(Y1) = +54.62 - 0.86*A + 15.30*B$$

Figure 7: Result of % CPR in 1 hr. of all batches

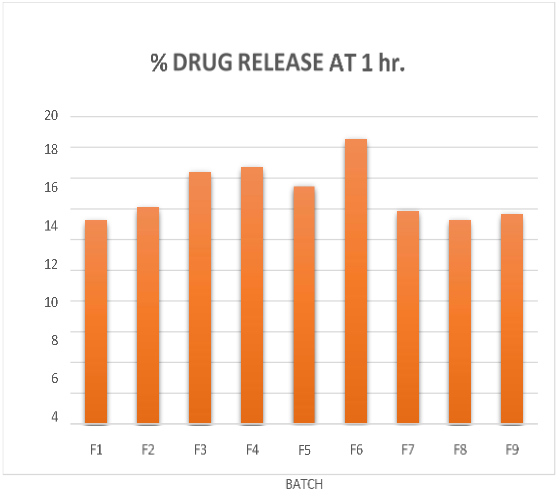


Figure 8: Result of % CPR in 5 hrs. of all batches

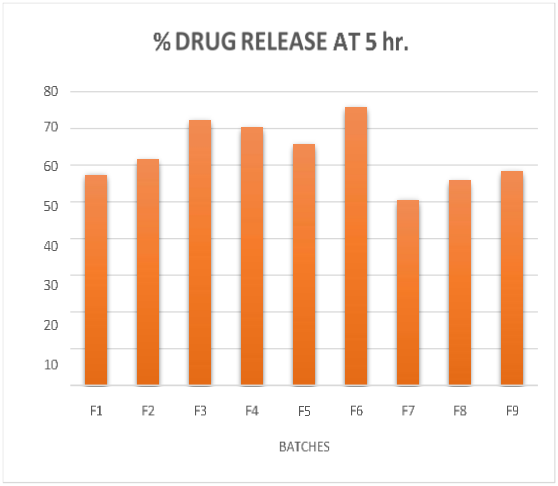


Figure 9: pH of all batches

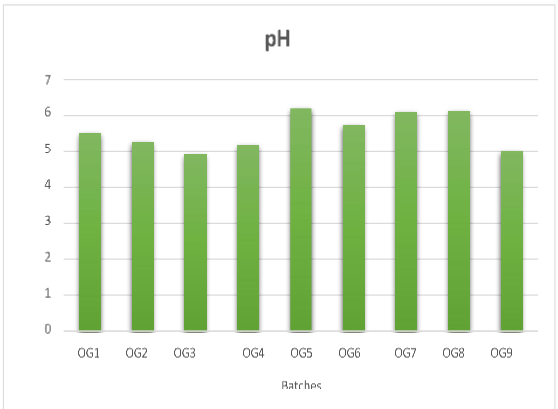


Table 1: 3^2 factorial design response factors

Batches	Coded value		Response factors			
	X1	X2	Y1(%OBC)	Y2(viscosity)	Y3(%CPR) (1 hr.)	Y4(%CPR) (5 hr.)
OG 1	-1	0	57.13	866.8	13.232	57.347
OG 2	0	1	68.8	1375	14.061	61.602
OG 3	0	0	55.26	910.30	16.393	72.227
OG 4	1	1	67.02	1230.1	16.704	70.236
OG 5	1	-1	35.13	500.02	15.466	65.544
OG 6	1	0	60.22	857.5	18.534	75.783
OG 7	-1	1	69.55	1032.21	13.847	50.465
OG 8	-1	-1	40.88	647.6	13.24	55.843
OG 9	0	-1	37.56	550.7	13.651	58.322

Table 2: Fitting of Statistics for % OBC(Y1)

	3.33	**R^2**	0.9550
Mean	54.62	**Adjusted R^2**	0.9399
C.V.%	6.09	**Predicted R^2**	0.8984
Press	149.87	**Adeq Precision**	16.8241

The R^2 value of Y1 were 0.9550 which indicates excellent correlation for all the batches

The Predicted R^2 of 0.8984 aligns closely alongside the modified R^2 of 0.9399, with a difference of less than 0.2. Adeq Precision measures the signal-to-noise ratio. A ratio higher than 4 is better. A ratio of 16.824 means that the signal is good. This model makes it easier to go about in the design area. [23]

Table 3: regression analysis of effect on A and B on Y1

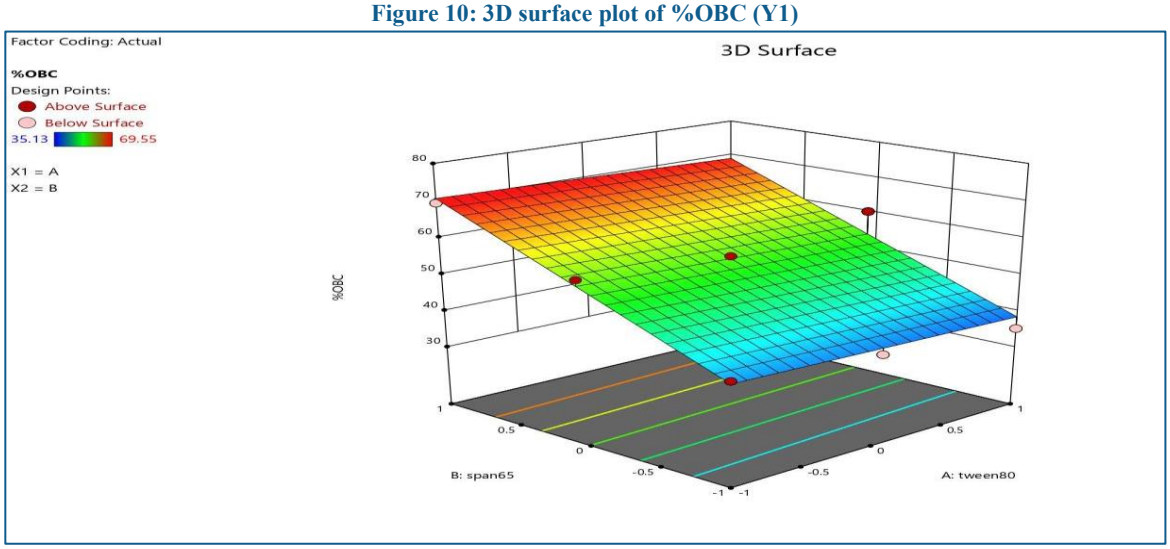
ANOVA for Response Surface Linear Model			
Factor	**Coefficient value**	**F value**	**P value**
Intercept	54.62	63.60	< 0.0001
A-tween80	-0.8650	0.4052	0.5479
B-span65	15.30	126.78	< 0.0001

The projected result is statistically significant, as shown by the model's F-value of 63.60. The probability that this F-value resulted from random noise is just 0.01%. When the P-value is below 0.0500, the predictive keywords indicate that the results are statistically significant. B is a crucial component of the model. The mathematical concepts lack significance if the values above 0.1000. Model reduction may be advantageous if your model is very long and lacks essential keywords necessary for preserving hierarchy.

2.1.1 Effect of independent variables on viscosity (Y2):

Viscosity Y2 The findings of the multiple linear regression examination indicated that coefficient A bear positive sign for coefficient value for X1 i.e. 6.84 and B bear positive sign for X2 i.e. 323.17. It indicates that X1 had minor positive effect on oil binding capacity and X2 had a major positive effect on oil binding capacity. Hence, we conclude that as the amount of span increased viscosity was increased. Although X2 had more effect on oil binding capacity than X1 variable.

Figure 10: 3D surface plot of %OBC (Y1)



The modified equation for response Y2 is shown below:

Viscosity(Y2) = +885.58 +6.84*A +323.17*B

Table 4: Fitting of statistics for viscosity(Y2)

Std. Dev.	109.56	**R^2**	0.8970
Mean	885.58	**Adjusted R^2**	0.8626
C.V.%	12.37	**Predicted R^2**	0.7230
Press	1936E+05	**Adeq Precision**	10.4341

The R^2 value of Y2 were 0.8970 which indicates good correlation for all the batches.

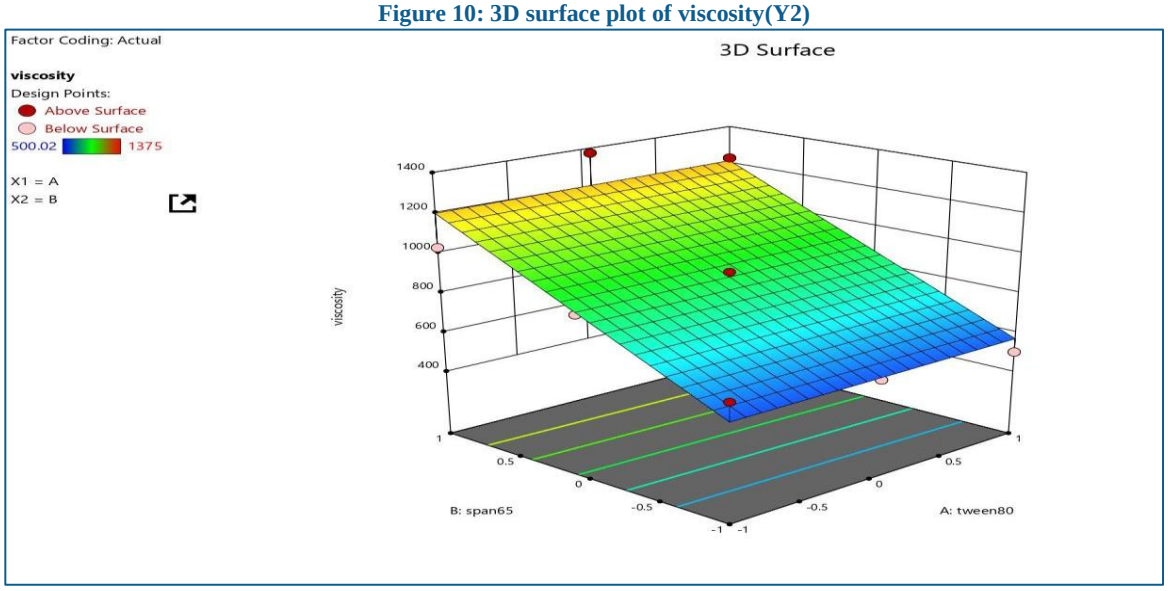
The Predicted R^2 of 0.7230 is quite close to the modified R^2 of 0.8626, alongside a variance of 0.2 or less. Adeq Precision measures the ratio of signal to noise. [24] A ratio higher than 4 is better. A ratio of 10.434 indicates that the signal quality is acceptable. This model makes it easier to go about in the design area.

Table 5: Regression analysis of effect of A and B on Y2

ANOVA for Response Surface Linear Model			
Factor	**Coefficient value**	**F value**	**P value**
Intercept	885.58	26.11	0.0011
A-tween80	6.84	0.0234	0.8836
B-span65	323.17	52.20	0.0004

The model's F-value of 26.11 shows that the hypothesis is of statistical significance. The chance of getting an F-value this high only by chance is just 0.11%. [25] If the p-values are less than 0.0500, it means that the predictive terms are of statistical significance. B is a key word in the model. Values higher than 0.1000 indicate that the predictive terms are not important. If your model has a lot of words that don't matter, model reduction could make it better, save for the ones that are needed to support hierarchy.

Figure 10: 3D surface plot of viscosity(Y2)



3.3.9.2 Effect of independent variables on in vitro drug release at 1 hr. (Y3):
%CPP Y3 The findings from the multiple linear regression examination indicated that coefficient A and B both bear positive sign for coefficient value for X1 and X2 i.e. 1.73 and 0.3758 respectively. It indicates that X1 and X2 both had a positive effect on drug release at 1 hour. [26] Although X1 had more effect on drug release than X2 variable as per equation.

The modified equation for response Y3 is shown below:

%CPR at 1 hr. (Y3) = +15.01-1.73*A+0.375*B

Table 6: Fitting of statistics for %CPR in 1 hr.

Std. Dev.	1.22	**R^2**	0.6770
Mean	15.01	**Adjusted R^2**	0.5693
C.V.%	8.15	**Predicted R^2**	0.3713
Press	17.48	**Adeq Precision**	5.9650

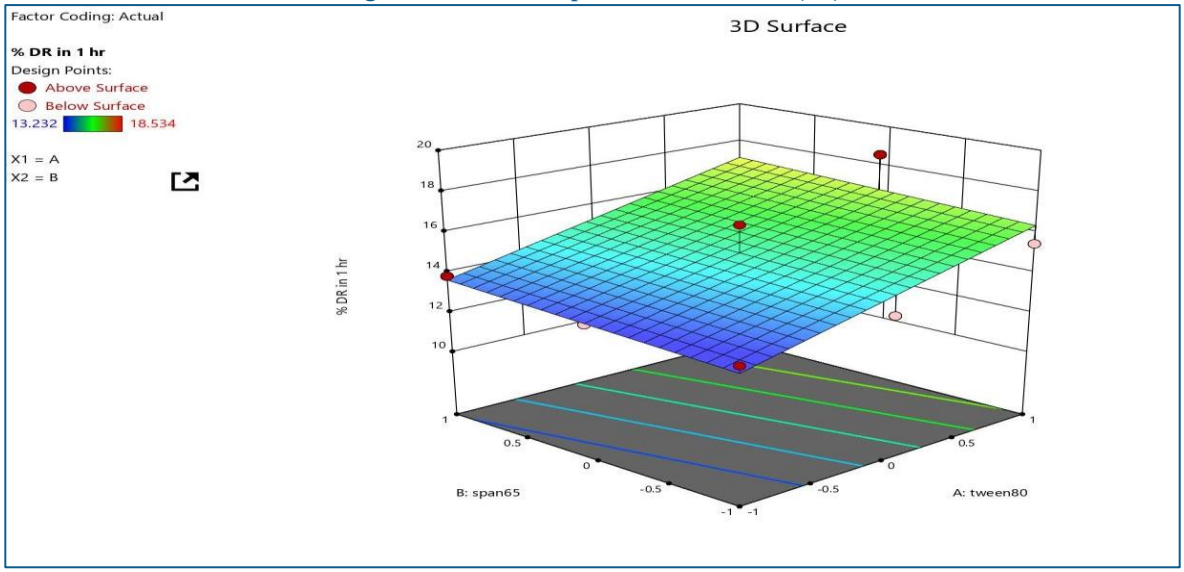
The modified R2 value of 0.5693, which is less than 0.2, is close to the 0.3713 predicted value. They compute the signal-to-noise ratio using Adeq Precision. The ratio should be more than 4. If the ratio is 5.965, the signal is sufficiently strong. Design experts have more mobility because to this framework. [27]

Table 7: Regression analysis of effect of A and B on Y3

ANOVA for Response Surface Linear Model			
Factor	**Coefficient value**	**F value**	**P value**
Intercept	15.01	26.11	0.0337
A-tween80	1.73	12.01	0.0134
B-span65	0.3758	0.5662	0.4802

The **model's F-value** of 6.29 shows how important it is. It is only 3.37% likely that noise could produce an F-value this big.
If the **P-value** is less than 0.0500, the model terms are essential. [28] In this case, the letter "A" is important. The model terms don't matter if the values are higher than 0.1000. Model reduction may make your model better by getting rid of terms that don't support the hierarchy and aren't significant.

Figure 12: 3D surface plot of %CPR in 1 hr.(Y3)



3.3.9.3 Effect of independent variables on in vitro drug release at 5 hrs. (Y4):
%CPP Year 4 The results of the multiple linear regression analysis indicate that coefficients A and B both exhibit positive values for X1 and X2, specifically 7.98 and 0.4323, respectively. X1 and X2 both positively influenced drug release at the 5-hour mark. X1 demonstrated a greater impact on drug release compared to the X2 variable, according to the equation. [29]

The modified equation for response Y3 is shown below:

$$\%CPR \text{ at } 5 \text{ hrs. } (Y4) = +69.46+7.98*A+0.43*B+2.52*A*B-1.51*A^2-8.12*B^2$$

Table 8: Fitting of statistics for %CPR in 5 hr.

Std. Dev.	2.95	**R^2**	0.9543
Mean	63.04	**Adjusted R^2**	0.8780
C.V.%	4.68	**Predicted R^2**	0.5854
Press	236.97	**Adeq Precision**	10.8578

The 0.2 variation between the Adjusted R^2 of 0.8780 and the Predicted R^2 of 0.5854 indicates a considerable divergence. This indicates a significant issue with the data and/or model, or a substantial obstruction. Considerations include model reduction, response transformation, and the existence of outliers. Confirmation runs are essential for verifying the functionality of all empirical models. Adeq Precision indicates the strength of the signal relative to the noise level. A ratio over 4 is preferable. When the ratio reaches 10.858%, the signal level is deemed satisfactory. This method facilitates exploration inside the design domain.

Table 9: Regression analysis of effect of A and B on Y4

ANOVA for Response Surface Quadratic Model			
Factor	**Coefficient value**	**F value**	**P value**
Intercept	69.46	12.52	0.0319
A-tween80	7.98	43.89	0.0070
B-span65	0.4323	0.1287	0.7436

The Model F-value of 12.52 indicates the statistical significance of the model. The probability of obtaining an F-value of this size due to random fluctuation is 3.19%. P-values < 0.0500 indicate that the model terms possess statistical significance. [30] A and B^2 are essential variables in this model. Values over 0.1000 indicate that the model terms lack statistical significance. Model reduction enhances your model by removing extraneous words while preserving those critical for sustaining hierarchy.

Figure 13: Contour plot effect X1 and X2 on %CPR in 5 hr.

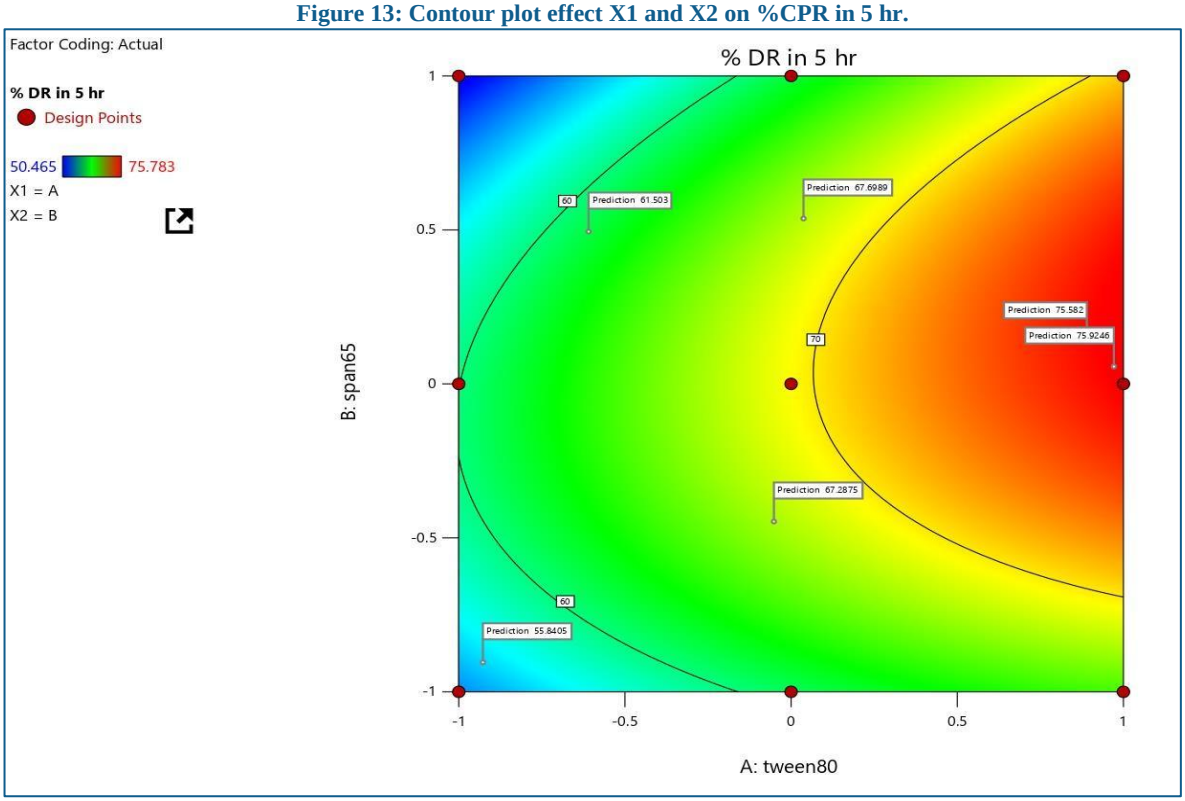
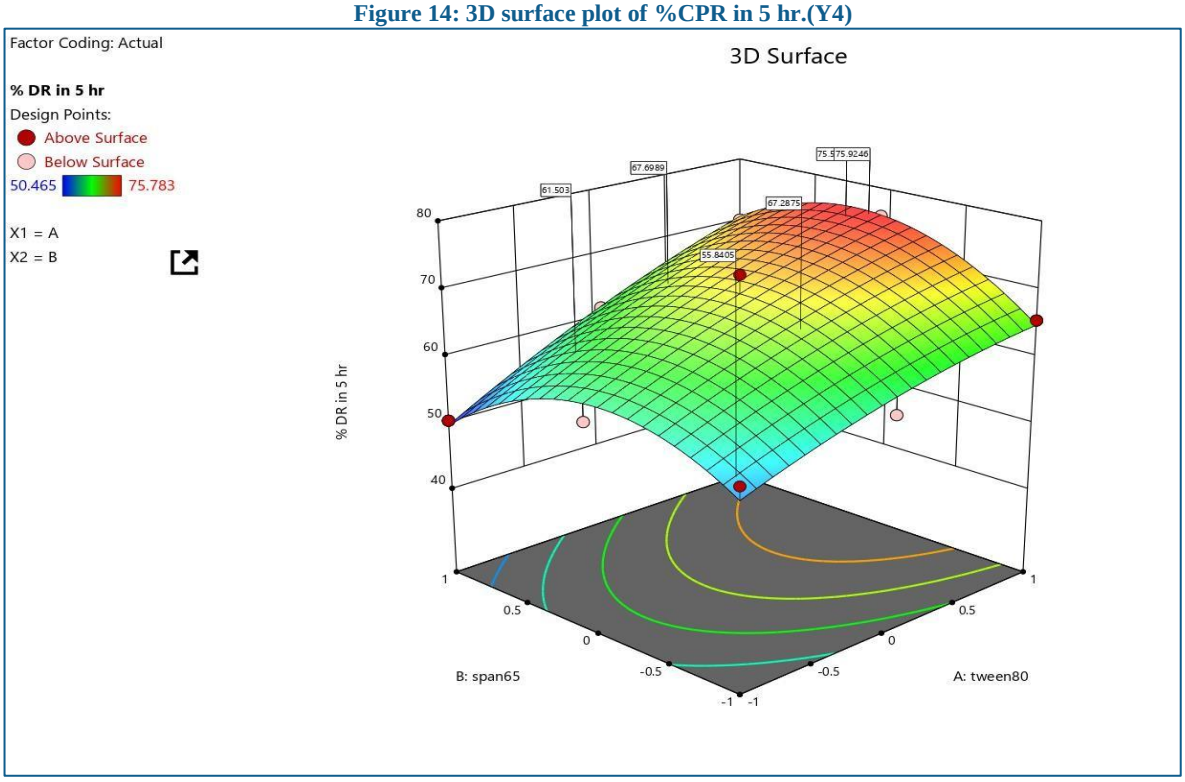


Figure 14: 3D surface plot of %CPR in 5 hr.(Y4)



3.3.9.4 Curated selection of an optimized batch

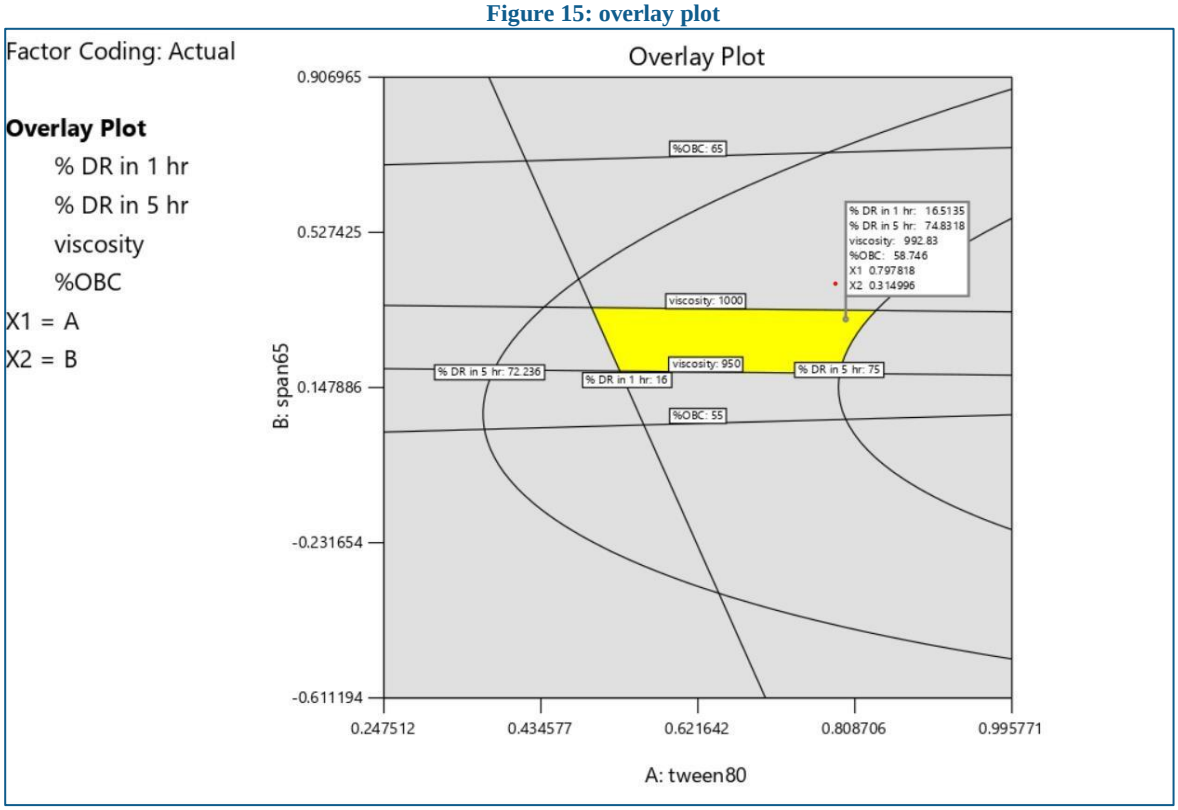
The ideal batch was determined through the application of Design Expert Software [version 13.0.5.0 statease]. The process of optimization was realized by employing the desirability function, which synthesizes all responses into a unified metric and enables the forecasting of ideal values for the independent variables. [31] The approach was utilized to evaluate the appeal of each of the dependent variables as well as the collective appeal by calculating the geometric mean. The batch exhibiting the highest overall desirability value, nearing 1, ought to be considered the optimal selection. The values chosen for the dependent variables Y1, Y2, Y3, and Y4 are displayed in the table.

Table 10: Desired criteria for dependent variables

Dependent variable	Desirable criteria	
	Lower limit	Upper limit
Y1 (%OBC)	55%	65%
Y2 (Viscosity)	950	1000
Y3 (In vitro drug release at 1 hr.)	16%	20%
Y4 (In vitro drug release at 6 hrs.)	72%	75%

From the data given in software the software will overlap the plots generated for all responses and it will give the area or design space, which will suggest the area in which any formulation prepared using specified values of factors X1 and X2 the results will be of desired range. The overlay plot is shown in **Figure 15** an example of formulation is shown in the same graph and the same batch is prepared for validation of design and the results are compared with that values which given by software.

Figure 15: overlay plot



The yellow portion of the plot denotes the area in which all selection criteria are met.

The X1 & X2 having 0.8 and 0.3 levels are considered as an optimized batch. The optimized batch was further identified by checkpoint batch evaluation.

3.3.9.5 Validation of Check Point Batch

In order to assess the reliability of the equations that describes the influence of the factors on the responses one check point batch was formulated. Composition of check point batch is shown in Table 11.

Table 11: validation of check point batch

Independent variable	Coded value	Actual value
X1 (Tween80)	0.79	9
X2 (Span65)	0.31	7

Check point batch was formulated as per parent formula and their responses were measured and mentioned in Table 12.

Table 12: Results of check point batch

Responses	Results
Y1- %OBC	58.74
Y2- Viscosity	992.83
Y3- %CPR at 1hrs	16.51
Y4- %CPR at 6hrs	74.83

2.1.2 Comparison of Results of Check Point Batch with Predicted Value

Table 13: Comparison of results of checkpoint batches with theoretical value

Responses	Results		
	Predicted value	Experimental value	% Prediction error
Y1	58.74	60.22	2.37
Y2	992.83	857.5	0.01
Y3	16.51	18.534	1.10
Y4	74.83	75.783	2.5

The results shows that there was no significant difference between predicted value which obtained from design expert software and experimental value for each variable i.e.; oil bindingcapacity, Viscosity and In vitro drug release at 1 hr. and 5 hrs. Predictionvalue must be not higher than 5%. Therefore, it can be concluded that this model was validated and fitted for this 3^2 factorial design.

2.2 Release kinetic of the optimized batch

Table 14: Results of release kinetic of optimized formulation

Parameters	Kinetic Models				
	Zero order	First order	Higuchi	Korsemeyer-Peppas	Hixon
R^2 value	0.8521	0.8875	**0.9793**	0.9511	0.876

Table 14 presents the coefficient value (R2) obtained for various kinetic simulations of drug release. The optimal model was chosen based on relatively high values of the correlation coefficient (R^2). In conclusion, the optimal model for drug release from the optimized oleogel aligns with Higuchi Model kinetics, suggesting that the oleogel can serve as a matrix for controlled release, a characteristic frequently associated with solid fibre oleogels.

3.3.9.5 Comparative Study of Prepared In-House Cefuroxime Loaded Gel and Prepared Oleo Gel Formulation of Cefuroxime:

The comparison of various parameter of optimize batch with the prepared in-house cefuroxime loaded hydro gel. Examination of the in vitro release of drugs for the optimized batch (F6) and hydro gel was carried out using Franz diffusion cell and it was found that the optimized batch and hydro gel shows 75.78% and 54.55% CPR respectively, hence we can conclude that formulation F6 shows higher penetration than that of hydro gel.

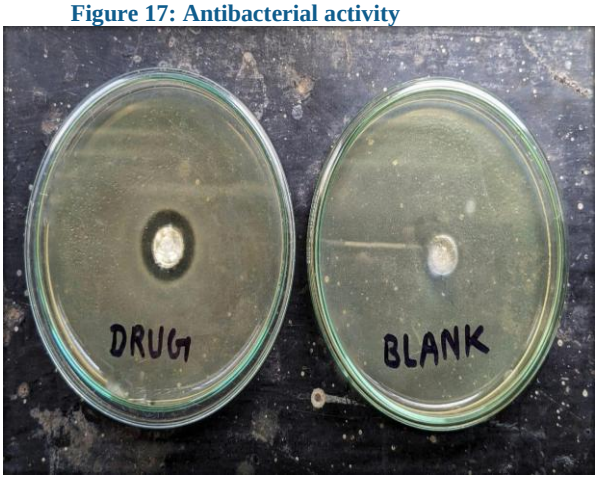
The faster drug release from oleogels compared to hydrogels is attributed to the solubility and partitioning advantages for lipophilic drugs, weaker matrix-drug interactions, and the less viscous and non-swelling nature of oleogels which facilitate easier drug diffusion.

Table 15: Comparative study of oleo and hydro gel

No.	Parameter	Oleo gel	In house prepared gel
1.	%CPR at 5 hr.	75.78	54.55
2.	pH	5.73	6.06
3.	Viscosity	857.5	1032
4.	Spreadability	22.17	35.67

Figure 16: Hydro gel and Oleo gel

Figure 17: Antibacterial activity



3.4 Antibacterial study

The oleogel loaded with drug (cefuroxime - 1% w/w) served as the active sample (A) whereasthe oleogel without drug acted as a control sample (B) for the antibacterial efficacy of the formulation against gram positive bacteria. The antibacterial activity following the incubation period is illustrated in Figure 17, and the results are presented in Table 16. The sample A demonstrated a zone of inhibition after 24 hours. Conversely, sample B exhibited no zone of inhibition. The findings indicate that the Cefuroxime axetil-loaded oleogel exhibits antibacterial activity.

Table 16: Results of zone of inhibition

Drug	Zone of inhibition (diameter; cm)	
	Active sample (A)	Control sample (B)
Cefuroxime,1% (w/w)	1.5 ± 0.2	Nil

3. 5Accelerated Stability study

This study aids in predicting the mechanisms underlying the destabilization of samples, which may occur due to alterations in their physico-chemical properties under extreme conditions. Oxidative changes are more probable at elevated temperatures, potentially affecting The viability of the samples is assessed. At lower temperatures, the formation of solid structures may change the interactions among the components of the sample, thus promoting the creation of interconnected patterns. This approach indicates the potential stability of a given entity. The samples must endure a minimum of five freeze-thaw cycles to be deemed stable. The optimized oleogel demonstrated stability for over 16 cycles, exhibiting no signs of instability.

Evaluation after 16 freezing-thawing cycles:

Table 17:
Results of optimized oleogel for Stability studies

Parameters	Before freezing-thawing cycles	After 16 freezing thawing cycles
Color of gel	Yellowish	Yellowish
Phase separation	NO	NO
Transparency	Hazy	Hazy
Consistency	Good	Good
Homogeneity	Homogenous	Homogenous
Spreadability (gcm/sec)	13.60	13.02
Oil binding capacity (%)	65	62
Viscosity (Cp)	910	900

Conclusion:

We successfully developed an oleogel comprising Span 65 as an oleogelator and Tween 80 as a co-oleogelator, which exhibited wound healing action. The CGC of Span 65 was determined to be 16% in sunflower oil. The FTIR research demonstrated that there is no interaction between Cefuroxime and the other excipients. The elevated concentration of Tween (80), the moderate concentration of Span (65), and a constant oil volume of 25 ml were designated as the optimal oleogel parameters. The oil binding capacity rose with the augmentation of Span 65, but a rise in oil quantity resulted in a drop in oil binding capacity. The gel-sol transition temperature (Tg) was determined to be contingent upon the concentration of the oleogelator. Viscosity is inversely related to the quantity of oil and directly related to the quantity of Span65. The in vitro drug release of the improved oleogel demonstrated a 75.34% drug release within 5 hours. The drug's release kinetics from the optimized oleogel adhered to Higuchi Model kinetics, suggesting that the oleogel may serve as a matrix for a controlled release system. The antibacterial investigation conducted using cefuroxime-loaded oleogel indicated that the formulation had antibacterial activity and may be used as a wound healing oleogel. Stability studies indicated that Oleogel remained stable after 16 freeze-thaw cycles.

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