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Formulation and Evaluation of Polyherbal Emulgel

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Abstract: The foible of allopathic medicines has resulted in adopting herbal plants that have been proven cost-effective with fewer adverse effects. Thus, the evaluation of the potential of herbal plants for the formulation of innovative dosage forms has resulted in the cure of various disease conditions. Thus, in context, the main aim of this work was to formulate the topical emulgel using herbal extracts of *Ocimum sanctum*, *Rubia cordifolia*, *Glycyrrhiza glabra* and *Punica granatum*. Emulgel was prepared by use of 23 factorial designs and the influence of the type of the gelling agent on viscosity and drug release from the prepared emulgel was investigated. The results found that the EG7 formulation was the optimized batch with pH of 6.3±0.02, viscosity of 5998.7±1.2 mPas, drug release of 89.75± 3.5% and in terms of stability. The results indicated the emulgel formulations were successful concerning all of the parameters.

Introduction

The Wound healing is the process by which a live creature restores its damaged or missing tissue by creating new tissue at the site of the injury. When the skin is healthy, the epidermis (the top layer) and the dermis (the middle layer) work together to protect the body from harmful environmental things. The barrier breakdown triggers a series of metabolic reactions that restore the harm (Gupta, 2022; Rieger et al., 2015).Its progression can be broken down into four distinct stages: haemostasis (blood clotting), inflammation, tissue expansion (cell proliferation) and finally, tissue remodelling (maturation & cell differentiation). Somewhat than being a different phase, blood clotting may be thought of as occurring during the inflammatory phase (Stadelmann et al., 1998). Because of its complexity and fragility, the wound-healing procedure can be disrupted or fail, resulting in the development of chronic wounds that refuse to heal. Chronic wounds that don't heal can be caused by many conditions, including diabetes, venous and arterial disease, infections, and the metabolic abnormalities that come with old age. Care for wounds, which includes cleaning and protecting the site from further injury or infection, promotes and expedites recovery (Rogers et al., 2017). First aid to complex nursing specialisations like wound, ostomy, continence care, and burn canter management all fall under this umbrella. Extracting, distilling, expressing, fractionating, purifying, concentrating, and fermenting herbal compounds and comminuting or powdering them can yield useful medicinal products. Emulgel formulation of the herbal extract was prepared by using liquid paraffin olive oil as the oil phase, Carbopol 934 as gelling agents, tween 80 as surfactants and methyl paraben/propyl paraben as preservatives (Versteeg et al., 2013; Umadevi et al., 2018).

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Materials and Methods

Chemicals

Amsar Pvt Ltd. supplied us *Ocimum sanctum, Rubia cordifolia, Glycyrrhiza glabra, and Punica granatum.* Research Fine Lab provided the liquid paraffin, Carbopol 934, and Tween 80, while Loba Chemical Pvt Ltd supplied the Span 80.

Software

Design Expert 13.0: Micro Math Inc., USA was used to formulate polyherbal topical emulgel.

Experimental design

First, the preliminary trials were done to determine the main factors and their concentrations. Among all the excipients, three factors liquid paraffin, carbopol 934 and tween 20 were selected as the independent factors. These dependent factors selected were viscosity and drug release. The concentrations of independent variables (as mentioned in Table 2) were selected through preliminary screening.

Optimization of formulation

Depending on the outcome of the pilot tests, researchers implemented one of 2^3 different factorial designs to determine how each independent variable affected the dependent ones. The dependent factors and the independent variables used in the design are in Table no 1 & 2. Total of 8 experimental batches was obtained through this design.

Polyherbal emulgel's preparation

Base gel was prepared by adding 01 gm of carbopol-934 polymer to 50 ml of water and vigorously stirring for 5 minutes for uniform mixing. The mixture was kept undisturbed at room temperature for the next 24 hours. The hydroalcoholic extract was dissolved in 15 ml of ethanol and water solution (60%-40%) with constant stirring to get the solution. The above-prepared hydroalcoholic extract solutions were individually added into the carbopol-934 polymer solution and mixed well to get the emulgel. Oil Phase was prepared by adding span 20 in light liquid paraffin.

In contrast, the aqueous phase was prepared by dissolving tween 20 in purified water, later methyl and propyl paraben was mixed in propylene glycol and further mixing was continued by using a magnetic stirrer till uniform dispersion of the extracts and preservatives was obtained. Both the oil and aqueous phases were heated at 70-80^0 C with continuous stirring and cooled to room temperature. The uniform dispersion was tested by intermittent checking of the pH of the emulgel and sodium hydroxide was added to make the neutral pH. All polyherbal topical emulgels were subjected to physical evaluation tests, as mentioned in Table 4 (Kavitha et al., 2013; Khan et al., 2022).

Physical evaluation of polyherbal topical emulgel

Visual inspections were performed to ensure a high-quality standard in terms of colour, finish, and uniformity.

Appearance/color

Physical analysis of the prepared polyherbal emulgel was observed.

pH

The pH of the formulation's one percent aqueous solution was determined employing a temperature- and pH-calibrated digital metre.

Irritancy study

Male and female Wistar rats had a 1 cm^2 spot branded into their dorsal surfaces on the left side. The individual topical emulgel was applied on the marked area, and latency to Irritancy, Erythema, and Edema development (if any) was recorded. Thereafter, observation was made for 6 hours up to 24 hrs.

Viscosity

The produced polyherbal emulgel's viscosity was tested in triplicate at room temperature using a Brookfield Viscometer using spindle 50 and at a speed of 50 rpm.

Table 1. List of dependent and independent variables in 2^3 factorial designs

A. Independent factors	A	Concentration of Liquid paraffin
	B	Concentration of Carbopol 934
	C	Concentration of Tween 20
B. Dependent Factors	Y_1	Viscosity
	Y_2	Drug release

Table 2. Independent variables and their concentrations used for formulations

Sr. No.	Independent variables	Coded levels	
		-1	+1
1	Concentration of Liquid paraffin (ml)	1.0	7.5
2	Concentration of Carbopol 934 (gm)	0.5	1.5
3	Concentration of Tween 20 (ml)	0.5	0.7

Spreadability

Two standard-sized glass slides were used in the experiment. A Polyherbal emulgel was applied between the two slides, to make a 60 mm sandwich. Excess emulgel was removed from the slide surfaces, and the slides were firmly fastened to a stand. A 20 g weight was added to the upper slide, and the time it took for it to move 60mm under the weight's impact was measured.

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The experiment was performed three times to ascertain the mean time, and spreadability was calculated using a specified formula.

Spreadability = (Weight × Length) / Time

Table 3. DOE formulation Batches

Contents	EG1	EG2	EG3	EG4	EG5	EG6	EG7	EG8
Extract (gm)	1	1	1	1	1	1	1	1
Shatdhaut Ghruta (gm)	1	1	1	1	1	1	1	1
Liquid Paraffin (ml)	1	1	7.5	1	7.5	1	7.5	7.5
Carbopol 934 (gm)	1.5	0.5	0.5	1.5	1.5	0.5	0.5	1.5
Tween 20 (ml)	0.7	0.5	0.7	0.5	0.5	0.7	0.5	0.7
Propylene glycol (ml)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Methyl paraben (gm)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Propyl paraben (gm)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Span 80 (ml)	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Mentha oil (ml)	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Distilled water (ml)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Extrudability

The formulations (10 gms) were put into the normal collapsible aluminium tubes and the ends were crimped shut. Every tube's weight was recorded properly. The tubes were then sealed by clamping in between two glass slides. The slides were covered with a 500 g weight, and the cap was taken off. To ascertain the formulation's extrudability, the extruded amount was collected, weighed, and a percentage was computed. Over 90% extrudability was considered excellent, over 80% was considered good, and over 70% was considered fair.

In-vitro diffusion study

The drug release studies used Franz diffusion cell (25 ml cell volume). 1 gramme of the formulation was uniformly applied to a specific area of cellophane membrane. The receptor chamber was filled with a Phosphate Buffer (pH 7.4) solution that was agitated using a magnetic stirrer. 1.0 ml aliquots were collected and replaced with new buffer solution at suitable time intervals. After appropriate dilution, the drug content in the obtained samples was determined using a UV visible spectrophotometer at 270 nm. This allowed for time-dependent analysis of the drug efflux through the membrane.

Wound healing activity

Group I served as a normal control in the excision wound model group (No treatment). The rats were given diethyl ether anaesthesia for the experiment, and a full-thickness skin excision was performed on the afflicted area, resulting in a wound area of roughly 500 mm^2. The emulgel was applied topically once daily until the site healed completely, and wound closure and epithelization time were measured. On days 0, 5, 10, 15, and 21, the percentage of wound closure was measured until the wound had totally re-epithelialized, which was assessed by the lack of any remaining raw wound when the scar was removed. The animals were given the newly prepared medication solutions for 21 days (Saxena et al., 2022; Sohail et al., 2022).

Release Kinetics

Several ideas and mathematical models exist to describe how medicines are liberated from pharmaceutical formulations. It is possible to investigate the underlying mechanism of drug release by turning in vitro drug release data into kinetic models.

Stability study

A four-week physical stability test was conducted on the polyherbal emulgel at two different temperatures and relative humidity levels (250°C±20°C, 60% RH ± 5% and 400°C±20°C, 75 % RH ± 5%).

Results

Properties of the formulations

On visual inspection, the formulation features of the cream were a uniform greenish coloured semisolid, emollient in feeling, and irritation free (table 4). Table 5 shows pH, Viscosity, Spradability, Extrudability, Drug content (%), and Drug Release (%). The hypothesis has statistical significance since the F-value for it is 9.24. A big F-value is extremely unlikely to be caused by random chance; the probability is only 2.85%.

If the p-value for a model term is less than 0.05, then it is significant. Here, the model term B is particularly important. If the value of a model term is greater than 0.1, it is not important in the model. If your model has a large number of meaningless terms (except those necessary to support hierarchy), you may benefit from performing a model reduction.

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There is a larger-than-expected discrepancy between the Expected R^2 of 0.4958 and the Adjusted R^2 of 0.7794.

Table 4. Physical properties of Polyherbaltopical emulgel

Formulation Code	Parameters		
	Appearance/Color	After feel	Irritancy
EG1	Greenish	Emollient	Nil
EG2	Greenish	Emollient	Nil
EG3	Greenish	Emollient	Nil
EG4	Greenish	Emollient	Nil
EG5	Greenish	Emollient	Nil
EG6	Greenish	Emollient	Nil
EG7	Greenish	Emollient	Nil
EG8	Greenish	Emollient	Nil

Table 5. Factorial design variables with their responses

Formulation	Factor 1 (A)	Factor 2 (B)	Factor 3 (C)	pH	Viscosity (m.Pas)	Spradability (gm.cm/sec)	Extrudability (g/cm^2)	Drug content (%)	Drug Release (%)
EG1	-1	+1	-1	6.7± 0.02	16204.5± 1.2	22.33 ± 1.2	16.1± 1.2	82.57 ± 1.2	62.35 ± 1.25
EG2	-1	-1	-1	6.9± 0.03	8570.5± 1.2	24.33 ± 2.1	17± 2.1	86.15 ± 1.1	77.54 ± 2.75
EG3	+1	-1	+1	7.1± 0.03	8347.7± 1.2	21.33 ± 3.2	16± 3.2	88.35 ± 2.2	87.52 ± 3.10
EG4	-1	+1	-1	6.8± 0.02	11300.2± 1.2	19.33 ± 3.7	13± 3.7	84.57 ± 1.7	67.56 ± 6.65
EG5	+1	+1	-1	7.0± 0.03	15506.3± 1.2	17.33 ± 2.5	12.5± 2.5	85.25 ± 2.5	71.27 ± 2.45
EG6	-1	-1	+1	6.9± 0.02	6201.4± 1.2	25.33 ± 2.1	15± 2.1	82.56 ± 2.7	68.75 ± 3.45
EG7	+1	-1	-1	6.3± 0.02	5998.7± 1.2	27.33 ± 3.7	16± 3.7	91.05 ± 1.5	89.75 ± 3.50
EG8	+1	+1	-1	6.7± 0.02	15750.5± 1.2	17.33 ± 1.5	13± 1.5	78.45 ± 2.1	62.42 ± 3.47

± Mean value with standard deviation of three replicates

Table 6. Viscosity ANOVA analysis

Source	Sum of Squares	df	Mean Square	F-value	p-value	Source
Model	1.145	3	3.817	9.24	0.0285	significant
A-Liquid paraffin	1.383	1	1.383	0.3350	0.5937	
B-Carbopol 934	1.098	1	1.098	26.60	0.0067	
C-Tween 20	3.288	1	3.288	0.7963	0.4226	
Residual	1.652	4	4.129			
Cor Total	1.310	7				

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It's possible that these points to a fault with your model or data, or it could be an indication of a significant block effect. It is recommended to perform confirmation runs on all empirical models. The signal-to-noise ratio is evaluated using Adeq Precision. It's preferable to have a ratio higher than 4. With a ratio of 6.629, your signal is strong enough. The design space can be explored with the help of this model.

Table 7. R^2 value for viscosity Fit Statistics

Std. Dev.	2031.94	**R^2**	0.8740
Mean	10984.98	**Adjusted R^2**	0.7794
C.V. %	18.50	**Predicted R^2**	0.4958
		Adeq Precision	6.6290

Coded-factors-based final equation

Viscosity = +10984.98 +415.82 A +3705.40 B + 641.05C

Last equation is founded on actual factors

Viscosity = -815.89615 +127.94+7410.80+ 6410.50

The answer for a particular level of each factor can be predicted using the corresponding equation in regard to the actual factors.

The model is statistically important, with an F-value of 9.31. An F-value this high could only arise from random chance 2.82 percent of the time.

The significance of model terms is shown by a p-value below 0.05. Here, the model term B is particularly important. If a model term's assessment is superior to 0.1, it is not important in the model. If your model has many meaningless terms (except those necessary to support hierarchy), you may benefit from performing a model reduction.

The gap between the Expected R^2 of 0.4987 and the Adjusted R^2 of 0.7807 is larger than 0.2, which is not as near as one might think. There could be an issue with your model and/or data, or it could be an indication of a huge block effect. Minimizing models, transforming responses, identifying outliers, etc. are all factors to think about. Confirmation runs should be used to test all empirical models. The signal-to-noise ratio can be determined with Adeq Precision. It's preferable to have a ratio higher than 4. A sufficient signal is shown by your ratio of 8.606. The design space can be explored with the help of this model.

Equation in Terms of Coded Factors

Drug Release = +4.29+0.0553A -0.1005B – 0.0448 C

Equation in Terms of Actual Factors

Drug Release = +4.684 + 0.0170A – 0.2010 B – 0.4479 C

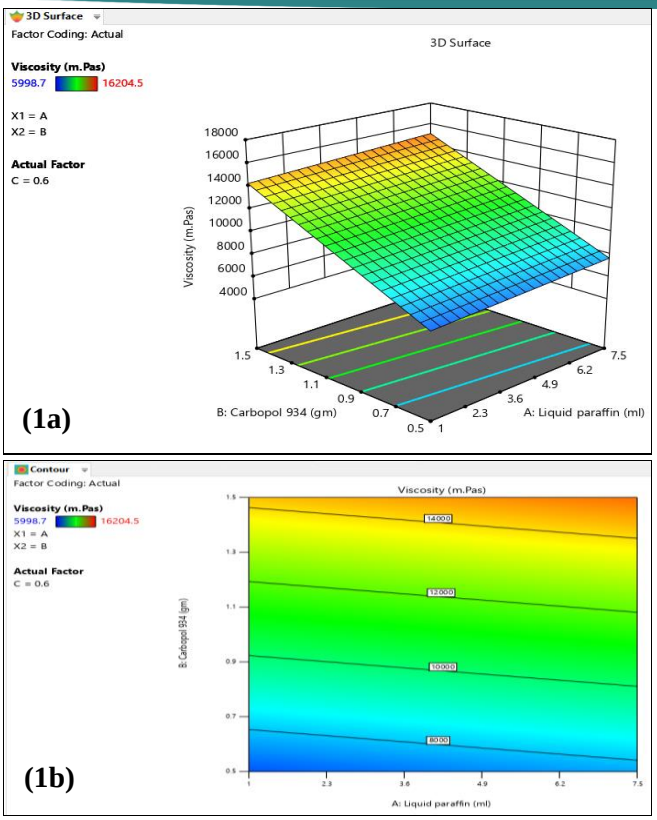

Figure 1.(a& b). 3-Dimensional response surface plots and contour plot for the viscosity of topical emulgel

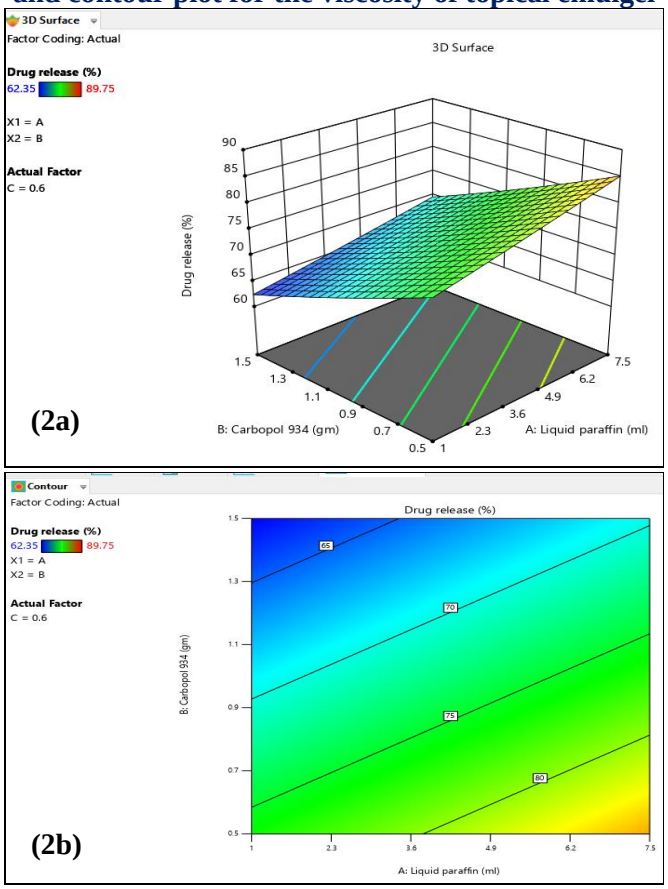

Figure 2. (a & b). Three-Dimensional response surface plots and Contour plots for drug release of topical emulgel.

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Table 8. Response 2 Drug release.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.1213	3	0.0404	9.31	0.0282	significant
A-Liquid paraffin	0.0244	1	0.0244	5.63	0.0767	
B-Carbopol 934	0.0808	1	0.0808	18.60	0.0125	
C-Tween 20	0.0160	1	0.0160	3.69	0.1270	
Residual	0.0174	4	0.0043			
Cor Total	0.1387	7				

Table 9. Fit Statistics

Std. Dev.	0.0659	**R^2**	0.8747
Mean	4.29	**Adjusted R^2**	0.7807
C.V. %	1.54	**Predicted R^2**	0.4987
		Adeq Precision	8.6061

Table 10. The Stability study of polyherbal emulgel formulations at 25°C ± 2°C/60%

Polyherbal emulgel	pH	Viscosity (mPas)	Spread ability (cm/s)	Extrudability	Drug release
EG1	6.7±0.02	16204.5±50	18±0.03	64±4.6	62.35±0.91
EG2	6.9±0.03	8570.5±45	16±0.02	81.2±5.6	77.54±1.35
EG3	7.1±0.03	8347.7±35	14.2±0.45	80±4.3	87.52±1.25
EG4	6.8±0.02	11300.2±43	15.5±0.14	74.2±5.3	67.56±1.10
EG5	7.0±0.03	15506.3±78	13.0±1.42	72±3.1	71.27±0.65
EG6	6.9±0.02	6201.4±48	16.5±0.09	78±4.6	68.75±2.05
EG7	6.3±0.02	5998.7±69	14.5±0.03	92±5.6	89.75±0.02
EG8	6.7±0.02	15750.5±61	14.5±0.11	71±4.6	62.42±0.37
± Mean value with standard deviation of three replicates					

Table 11. The Stability study of polyherbal emulgel formulations at 40°C ± 2°C/75% RH ± 5%

Polyherbal emulgel	pH	Viscosity (mPas)	Spread ability (cm/s)	Extrudability (Pa)	Drug release
EG1	6.5±0.01	16201.7±50	18±0.01	64±4.5	62.32±0.80
EG2	6.7±0.02	8570.3±42	15±0.02	80.2±5.5	75.55±1.32
EG3	7.0±0.01	8345.5±37	14.1±0.47	81±4.2	86.37±1.15
EG4	6.7±0.02	11302.1±42	15.5±0.14	75.2±5.2	65.70±1.07
EG5	6.8±0.03	15505.2±75	13.0±1.41	71±3.0	71.25±0.55
EG6	6.9±0.01	6202.5±45	15.5±0.07	75±4.5	67.85±2.15
EG7	6.3±0.02	5997.5±65	14.7±0.02	93±5.7	89.70±0.11
EG8	6.2±0.03	15747.7±62	14.5±0.10	72±4.5	62.50±0.25
± Mean value with standard deviation of three replicates					

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Wound healing activity

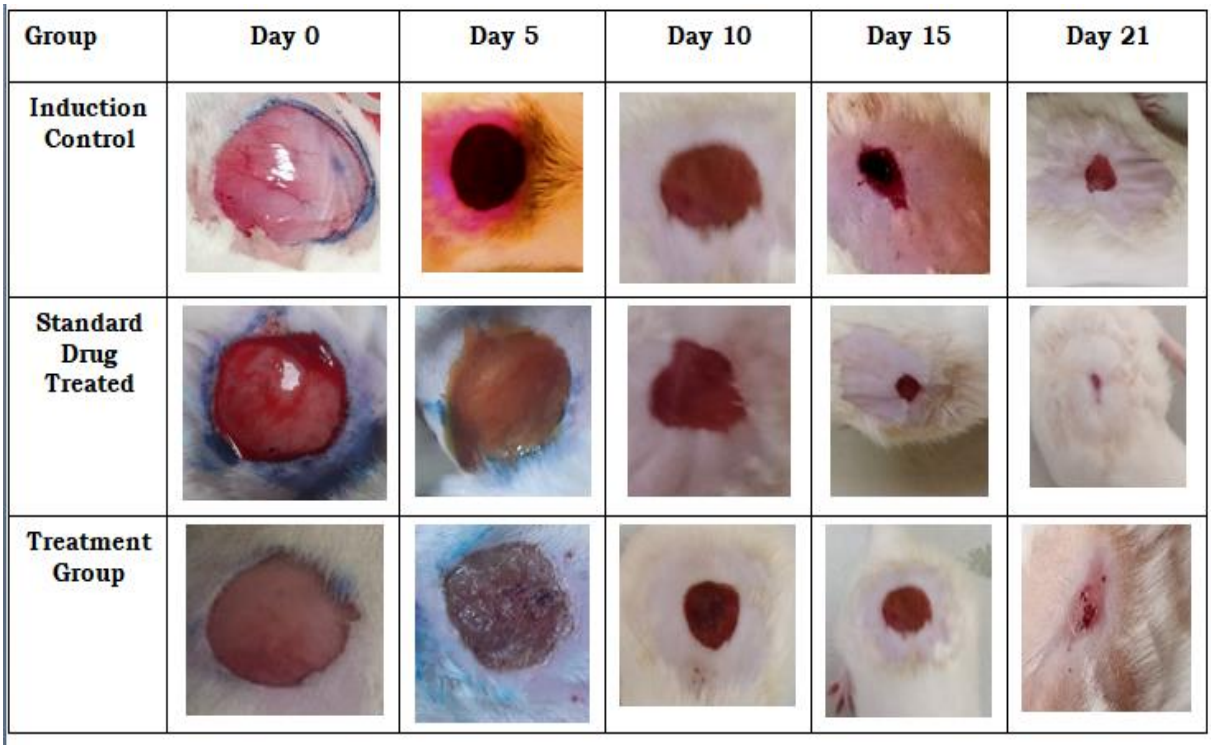

Figure 3.Pictures of the wound healing process at various time points in a rat model of an excision wound

Table 12. Effects of emulgel formulation on excision wound model wound contraction and epithelialization time for optimal batch

Group	% wound contraction				Epithelialization period (days)
	5th day	10th days	15th days	21st days	
Group I untreated	5.18±0.929	31.18±0.947	34.70±2.306	49.05±4.347	29.5±0.5
Group II standard	4.22±0.891^{ns}	31.18±1.709^{ns}	65.58±2.268***	75.58±4.856***	25.75±1.1
Group III Emulgel formulation	1.63±0.947^{ns}	34.44±2.751**	49.62±5.697^{ns}	74.38±2.833***	27.25±1.32

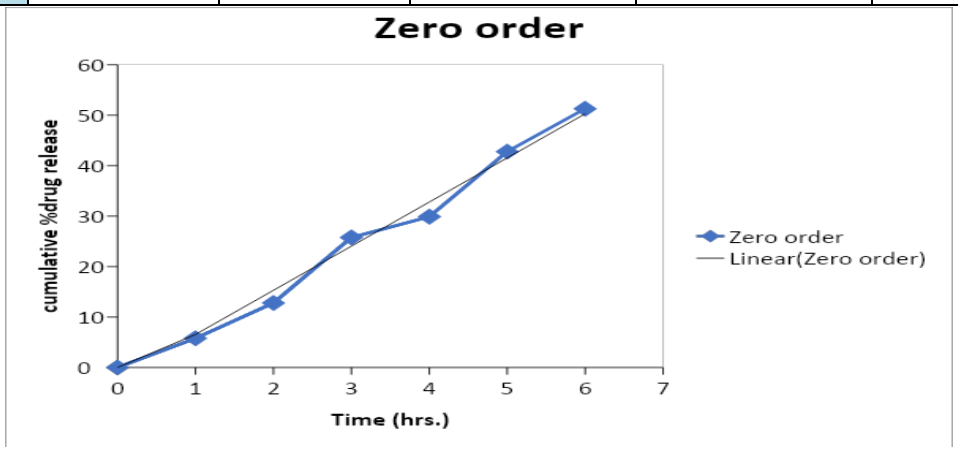

Figure 4.Zero-order drug release kinetics for optimized formulation

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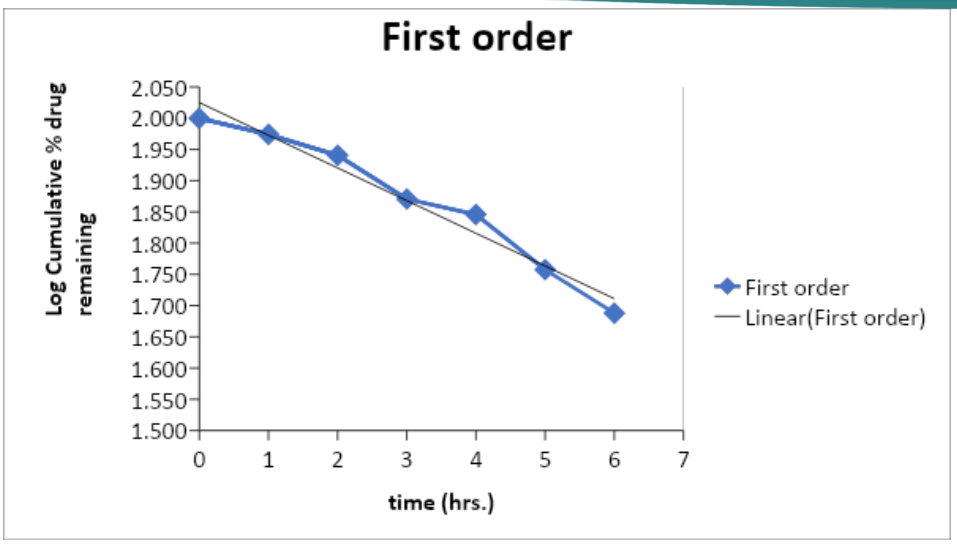

Figure 5. First-order drug release kinetics for optimized formulation

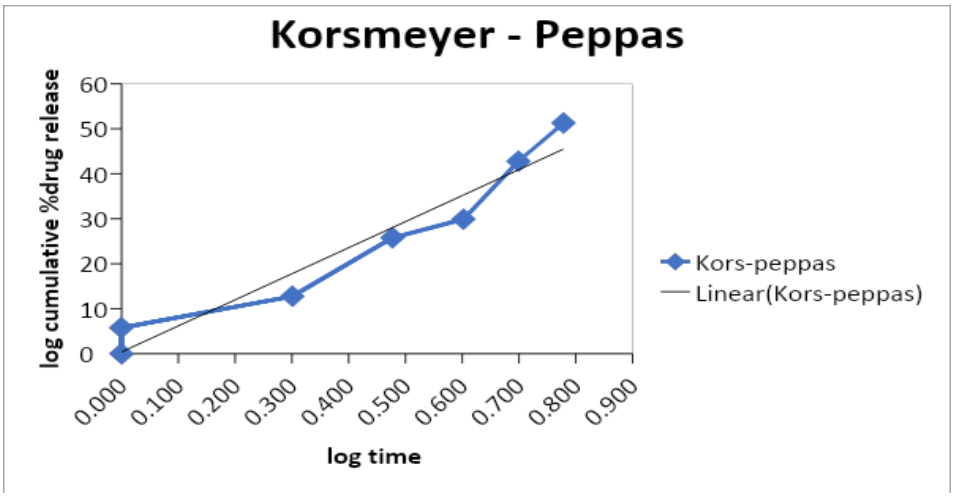

Figure 6. Korsmeyer- Peppas drug release kinetics for optimized formulation

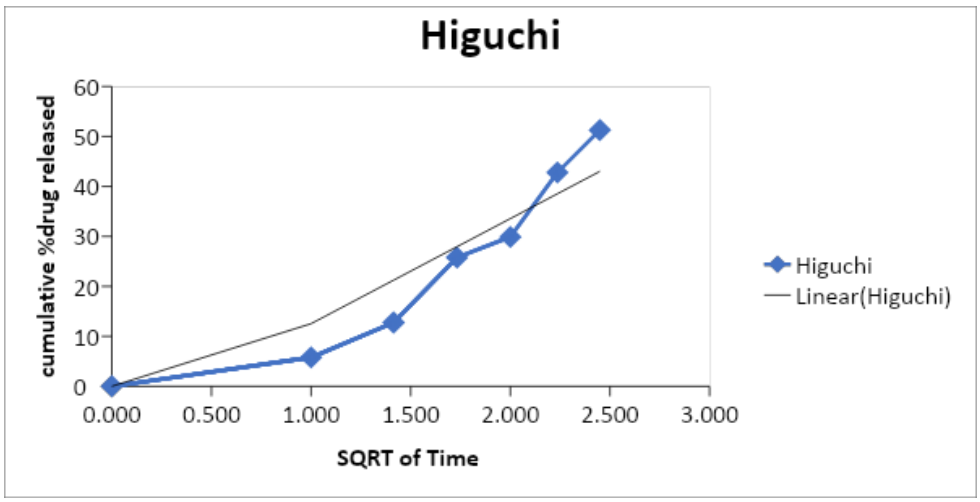

Figure 7. Higuchi model drug release kinetics for optimized formulation

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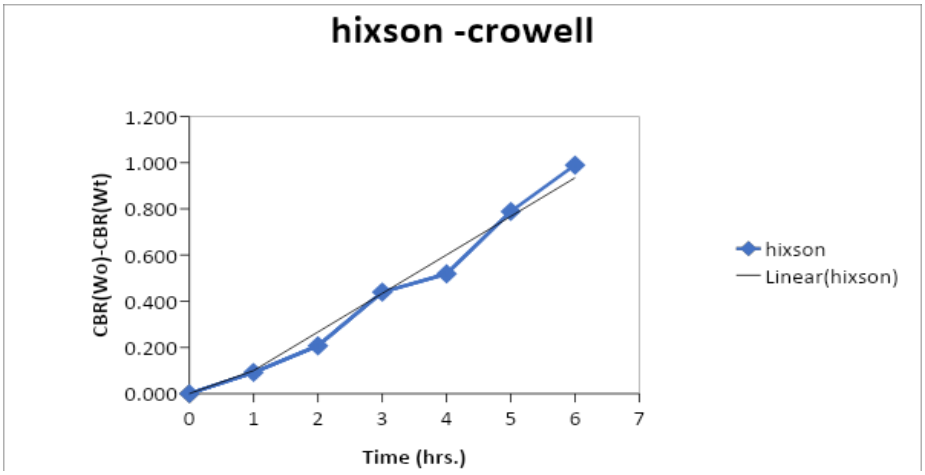

Figure 8. Hixson-Crowell model drug release kinetics for optimized formulation

Table 12. The regression coefficients obtained from model fitting

MODEL	Linear Regression Coefficient(R^2)	K Value
Zero-order	0.988	8.74
First-order	0.968	0.05
Korsmeyer and Peppas	0.941	57.93
Higuchi	0.864	21.02
Hixon-Crowell	0.977	0.16

The answer for a particular level of each factor can be predicted using the corresponding equation in terms of actual factors.

Kinetics of drug release

Several kinetic models were used to fit *in vitro* release data from different formulations, allowing researchers to learn more about the kinetics and mechanism of drug release.

Discussion

All stability parameters were met, and the formulations' viscosity values were within acceptable ranges without any noticeable change in homogeneity. Viscosity tests revealed that batch EG8 had superior viscosity characteristics than other formulations. After making the polynomial equations that show how the dependent and independent variables are related, the method was tweaked to get the best answers. Based on the outcomes of the analyses, the optimal formulation was chosen (Rajad et al., 2023; Chellathurai et al., 2023).

As a result, E7 was the best possible formulation, with a pH of 6.3±0.02, viscosity of 5998.7±1.2 mPas, drug release of 89.75± 3.5% (table 11).

The release data was fit to several kinetic models, including one that plots the cumulative percentage of drug release against time (a zero-order kinetic model) (figure 4), another that plots the log cumulative percentage of drug remaining against time (a first-order kinetic model) (figure 5), and yet another that plots the cumulative percentage of drug release against the square root of time (Higuchi model) (figure 7) (Dubey et al., 2023; Rathi et al., 2022). Table 12 provides a tabulation of the R^2 values. All of the formulas provided the best fit to the kinetic data. In this case, zero-order release kinetics was the most accurate description of the emulgel's release. This can be seen by comparing the regression coefficients of the first-order, Korsmeyer-Peppas, Higuchi, and Hixson-Crowell models (figure 8), where R^2 was found to be 0.988. It was seen that the topical emulgel worked well as a preventive, which showed that it was an effective formulation (Tanimu et al., 2022).

Conclusion

This work is about making and testing topical emulgel with different concentrations of gelling agents like carbopol 934. All of the raw materials used are of standard grade. Optimized batch EG7 showed an ideal gelling agent in a concentration of 1.5 with good viscosity and spreadability and showed uniformity of emulgel content. So, the emulgel will store the drug and release it slowly over time. So, the optimised formula can be used to treat diseases that affect the skin.

Conflict of Interest

There is no known reported conflict of interest in this publication.

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