ORIGINAL ARTICLE

Engineering Cubosomes-Based Nanocarriers for Burn Wound Healing: Formulation and Optimization of a Triple-Drug Delivery System

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Abstract

Objectives: The present study was designed to prepare and optimize a cubosomal gel co-loaded with silver sulfadiazine (SSD), Vitamin D, and tretinoin for enhanced burn wound healing. **Materials and Methods:** The drugs' active pharmaceutical ingredients (APIs) were characterized for the preformulation study (organoleptic properties, melting point, ultraviolet [UV] spectroscopy, Fourier-transform infrared [FTIR] spectroscopy, and solubility). Cubosomes of SSD were prepared and optimized using a factorial design and response surface methodology (RSM) with particle size (PS), entrapment efficiency (EE), and polydispersity index (PDI) as dependent variables. The optimized cubosomal dispersion was combined into a topical cubosomal gel and characterized for physicochemical characteristics, *in vitro* drug release, rheological behavior, release kinetics, and *in vivo* wound healing activity using an excision burn wound model in rats and stability over 90 days. **Results:** Preformulation studies of API confirmed the purity of SSD and tretinoin, with melting points of 281–285°C and 178–182°C, respectively. UV and FTIR analyses of the drug confirmed characteristic absorption peaks without evidence of drug–excipient incompatibility. Optimize cubosomal (CF11, 2%) formulation showing a PS of 186.7 nm, PDI of 0.286, and EE of 85.33%. The formulation showed a negative zeta potential and definite cubic structure as observed by Field Emission Scanning Electron Microscopy. The cubosomal gel was stable, homogeneous, and skin compatible, with a pH range of 6.22–6.78 and pseudoplastic shear-thinning behavior. *In vitro* release studies of cubosomal gel formulation showed sustained drug release over 8 h, with cumulative releases of 93.7%, 87.3%, and 86.3% for SSD, Vitamin D and tretinoin, respectively. SSD release showed the Higuchi model ($R^2 = 0.9966$), whereas tretinoin and Vitamin D release were best fitted by the Korsmeyer–Peppas model ($R^2 = 0.9992$). Stability studies confirmed negligible changes in physicochemical parameters during 90 days of storage. *In vivo* burn wound studies exposed significantly greater wound contraction and faster healing in animals treated with the optimized cubosomal gel formulation compared with control and standard treatment groups. **Conclusion:** The optimized cubosomal gel of SSD, tretinoin, and Vitamin D confirmed favorable physicochemical properties, high drug EE, sustained release, suitable stability, and enhanced burn wound healing activity. This optimize formulation represents a capable multi-drug topical nanocarrier system for the effective management of thermal burn injuries.

Key words: Cubosomes, formulation optimization, silver sulfadiazine, sustained release, thermal burn, topical gel, wound healing

INTRODUCTION

Burn injuries are among the most distressing forms of trauma, triggering significant morbidity and mortality worldwide.[1] Thermal burns outcome from exposure to excessive heat and lead to different degrees of tissue damage, ranging from superficial epidermal injury to extensive destruction of the skin and underlying tissues.[2] The skin serves as the body's primary protective barrier against microbial invasion and dehydration; therefore, disruption of this barrier following a burn injury raises the risk of infection, delayed healing,

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fluid loss, and scarring.[3] Effective burn management needs rapid wound closure, infection control, modulation of inflammation, and promotion of tissue regeneration.[4]

Silver sulfadiazine (SSD) has been broadly employed as a standard topical antimicrobial agent for burns due to its broad-spectrum antibacterial activity against Gram-positive and Gram-negative microorganisms.[5] SSD reduces microbial colonization, which is major contributor to delayed healing and burn-related complications.[6] However, extended use of conventional SSD formulations may be associated with late epithelialization and recurrent dressing changes, necessitating the development of improved delivery systems that can offer sustained drug release and enhanced therapeutic outcomes.[7]

Tretinoin (all-trans retinoic acid), a biologically active derivative of Vitamin A, plays a significant role in skin repair and regeneration.[8] It promotes keratinocyte proliferation, angiogenesis, collagen synthesis, and re-epithelialization, thus accelerating wound closure and reducing scar formation.[9] Similarly, Vitamin D is progressively recognized for its beneficial effects on skin health and wound healing.[10] Vitamin D controls cellular differentiation, immune responses, and inflammatory pathways, while also enhancing the production of antimicrobial peptides.[11] The combined use of SSD, tretinoin, and Vitamin D therefore offers a complex therapeutic approach targeting infection control, immune modulation, tissue regeneration, in burn wounds.[12]

Despite their therapeutic potential, the clinical effectiveness of these drugs may be limited by poor aqueous solubility, inadequate skin penetration, instability, and rapid drug clearance from the wound site.[13] Nanotechnology-based delivery systems have developed as promising strategies to overcome these challenges.[14] Among them, cubosomes are nanostructured lipid carriers composed of bi-continuous cubic liquid crystalline phases, which are stabilized by surfactants.[15] Cubosomes possess unique structural characteristics, including a large internal surface area, bioadhesive properties, high drug-loading capacity and also encapsulate both hydrophilic and lipophilic drugs.[16] These characteristics permit controlled drug release, enhanced skin permeation, and prolonged retention at the site of application.[17]

Topical gels combining cubosomal nanoparticles provide additional advantages such as patient compliance, localized drug delivery, ease of application and improved residence time on the wound surface.[18] Furthermore, the combination of multiple therapeutic agents within a single cubosomal system may produce synergistic effects that enhance the overall wound-healing process.[19]

Therefore, the present study was assumed to develop and optimize a cubosomal gel co-loaded with SSD, tretinoin, and Vitamin D for the management of thermal burn wounds. A factorial design and response surface methodology (RSM) were employed to optimize the formulation with respect to particle size (PS), entrapment efficiency (EE), and polydispersity index (PDI).[20] The optimized cubosomal gel was evaluated for its physicochemical characteristics, drug release behavior, stability, and *in vivo* burn wound healing efficacy.[21] The study aimed to establish a novel multi-drug nanocarrier system capable of providing sustained release, enhanced therapeutic efficacy, and improved healing outcomes in thermal burn injuries.

MATERIALS AND METHODS

SSD was purchased from SRL Pvt. Ltd., Taloja. Glyceryl monooleate (GMO) was purchased from Chemdyes Corporation. Poloxamer 407 was kindly gifted by BASF India. Polyvinyl alcohol (PVA), Carbopol 934, ammonia solution, glycerol, methyl paraben, and methanol were obtained by Loba Chemie Pvt. Ltd. Spectra/Pore dialysis membrane (12,000–14,000 mw cutoff) was purchased from SK Traders Indore.

Preparation of cubosomes

Cubosomal dispersions were prepared based on emulsification of a monoglyceride–surfactant system in an aqueous phase.[22] The composition of cubosomal dispersion is presented in Table 1. GMO was melted at 70°C using a water bath.[23] Separately, Poloxamer 407 and PVA were dissolved in distilled water at 70°C.[24] The molten lipid phase was added dropwise into the aqueous surfactant solution under continuous magnetic stirring at 1500 rpm for 15 min.[25] The resulting mixture was further homogenized using a mechanical stirrer at 1500 rpm for 2 h.[26] The dispersion was then allowed to cool to room temperature.[27] Post-emulsification, the formulation was subjected to ultrasonication (5 pulses ON and 2 pulses OFF) for 15 min to reduce PS and improve uniformity.[28] The final cubosomal dispersions were stored at room temperature until further analysis.[29]

Optimization of cubosomes using design of experiments

Optimization of cubosomal formulations was achieved using a Quality by Design method employing the Box–Behnken Design (BBD).[30] SSD – loaded cubosomes were optimized by identifying critical formulation variables influencing PS, PDI, and EE.

Experimental design

A three-factor, three-level BBD was implemented using Design-Expert® software (Version 13, Stat-Ease Inc., USA).[31] This RSM design was selected due to its effectiveness in

evaluating quadratic interactions with a smaller number of experimental runs (n = 12).[32]

Independent variables

- X_1: GMO concentration (% v/v)
- X_2: Poloxamer 407 concentration (% w/v)
- X_3: PVA concentration (% w/v).

Each factor was evaluated at 3 levels: Low (−1), medium (0), and high (+1).[33]

Dependent variables

- Y_1: PS (nm)
- Y_2: PDI
- Y_3: EE (%).

Table 1: Composition of cubosomal formulations

S. No.	GMO (%)	Poloxamer 407 (%)	PVA (%)
1	15	1.25	2.5
2	25	0.5	2.5
3	25	1.25	0
4	5	2	2.5
5	5	1.25	5
6	15	0.5	0
7	25	2	2.5
8	5	0.5	2.5
9	15	2	5
10	15	0.5	5
11	25	1.25	5
12	15	2	0
13	5	1.25	0

GMO: Glyceryl monooleate PVA: Polyvinyl alcohol

The design enabled the generation of 3D response surfaces and contour plots to evaluate factor interactions and optimize formulation parameters.[34]

Characterization of SSD-loaded cubosomes

EE

EE was performed using the dialysis bag method.[35] 1 mL of cubosomal dispersion was placed in a dialysis membrane (MWCO 110) and immersed in phosphate buffer (pH 6.8) at 250–300 rpm for 3 h. The unentrapped drug was quantified spectrophotometrically at 254 nm.[36]

PS and zeta potential

PS and zeta potential were analyzed using photon correlation spectroscopy (Malvern Zetasizer 3000, UK) at 25°C.[37] Measurements were performed in triplicate.

Morphological evaluation

Optical microscopy (×400) was used for preliminary visualization.[38] For SEM, the sample was prepared by resuspending the pellet in Milli-Q water so as to make the cubosomal suspension and a drop of it was placed on a glass film and dried at room temperature in a desiccator. Before SEM examination, this dried sample was gold-coated to a thickness of 10 nm with a Q150R Rotary-Pumped Sputter Coater. The coated particles were then examined under Field Emission Scanning Electron Microscopy (FESEM) (SU8010 series Hitachi, Japan).[39]

Preparation of cubosomal gel (Cubogel)

Cubosomal gels were prepared using Carbopol 934 (1–3% w/w) as a gelling agent.[40] Carbopol was hydrated overnight and mixed with propylene glycol and

Table 2: BBD experimental results of cubosomes

Standard	Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
		A: GMO	B: P407	C: PVA	Particle size	EE	PDI
		% v/v	% w/v	% w/v	nm	%	
3	1	5	2	2.5	124.3	85.75	0.171
6	2	25	1.25	0	191	91.38	0.261
4	3	25	2	2.5	227.3	74.4	0.378
12	4	15	1.25	2.5	112.4	90.15	0.25
8	5	25	1.25	5	150.6	80.7	0.246
1	6	5	0.5	2.5	280.2	87.28	0.537
2	7	25	0.5	2.5	133.8	86.75	0.228
7	8	5	1.25	5	381.2	82.4	0.483
11	9	15	0.5	5	180.6	89.45	0.225
10	10	15	2	0	185.6	90.94	0.195
9	11	15	0.5	0	139.8	84.23	0.247
5	12	5	1.25	0	133.6	80.48	0.212

BBD: Box Behnken design

Table 3: pH of formulation CF2 (1%, 2%, and 3%) and CF11 (1%, 2%, and 3%)

S. No.	Formulation code (%)	pH
1	CF2 (1)	6.62
2	CF2 (2)	6.63
3	CF2 (3)	6.50
4	CF11 (1)	6.45
5	CF11 (2)	6.78
6	CF11 (3)	6.22

Table 4: Viscosity of formulation CF2 (1%, 2%, and 3%)

Formulation code (%)	RPM	Torque %	Viscosity (cp)
CF 2 (1)	10	21.3	51300
CF 2 (1)	20	18.3	27650
CF 2 (1)	50	23.7	14140
CF 2 (1)	100	19.6	8260
CF 2 (2)	10	25.2	31300
CF 2 (2)	20	24.9	16500
CF 2 (2)	50	16.6	8490
CF 2 (2)	100	19.1	5290
CF 2 (3)	10	24.4	24400
CF 2 (3)	20	25.6	13800
CF 2 (3)	50	19.7	7140
CF 2 (3)	100	20.3	4330

Table 5: Viscosity of formulation CF11 (1%, 2% and 3%)

Formulation code (%)	RPM	Torque %	Viscosity (cp)
CF 11 (1)	10	21.5	52400
CF 11 (1)	20	23.9	27950
CF 11 (1)	50	24.3	14230
CF 11 (1)	100	24.4	8960
CF 11 (2)	10	23.2	31900
CF 11 (2)	20	21.8	17100
CF 11 (2)	50	18.3	8580
CF 11 (2)	100	19.4	5450
CF 11 (3)	10	21.5	23900
CF 11 (3)	20	25.4	14100
CF 11 (3)	50	21.3	7250
CF 11 (3)	100	24.2	4360

glycerol under stirring. Optimized cubosomal dispersion was incorporated along with tretinoin and Vitamin D. Methyl paraben was used as a preservative, and pH was adjusted using triethanolamine.[41]

Evaluation of cubosomal gel

Visual appearance

Formulations were evaluated for color, texture, homogeneity, and phase separation.[42]

pH determination

pH of the sample was measured using a calibrated digital pH meter (LABTECH).[43] 1 g gel was dispersed in 10 mL of distilled water and equilibrated before measurement.

Viscosity measurement

Viscosity of formulation was determined using a Brookfield viscometer (spindle No. 6).[44] Measurements were taken at 10, 20, 50, and 100 rpm to evaluate rheological behavior.[45]

In vitro drug release study

Drug release of cubosomal formulation was performed using a Franz diffusion cell with dialysis membrane (MWCO 12,000–14,000 Da) at 37 ± 0.5°C.[46] Samples were analyzed at 254 nm for SSD and 348 nm, 270 nm (tretinoin and Vitamin D).[47]

Kinetic modeling

Release data were fitted into first-order, zero-order, Higuchi and Korsmeyer–Peppas models.[48] Best fit was selected based on R^2 values.

Stability study

Accelerated stability studies for the optimized cubosomal gel formulation were conducted in accordance with International Council for Harmonisation Q1A (R2) guidelines. The formulation was stored at 40°C ± 2°C/75 ± 5% relative humidity in a stability chamber, and samples were withdrawn at predefined time intervals of 0, 30, 60, and 90 days. At each time point, the formulation was evaluated for key physicochemical parameters, which included pH, viscosity, appearance, and % drug release to assess any changes indicative of physical or chemical instability.

In vivo burn wound healing study

Experimental design

Wistar rats were divided into four groups ($n = 4$):[49]

Group treatment

i. SSD cubosomes
ii. Povidone iodine (5%)
iii. SSD + Tretinoin + Vitamin D cubosomes
iv. Blank formulation.

Table 6: Different models of drug release

Formulation	Zero order	First order	Higuchi model	Korsmeyer–Peppas model	Release mechanism
CF11 (2%) (SSD)	0.9747	0.9675	0.9966	0.9886	Higuchi
CF11 (2%) (tretinoin)	0.9903	0.9684	0.993	0.9992	Korsmeyer–Peppas
CF11 (2%) (Vitamin D)	0.9944	0.9566	0.9887	0.9992	Korsmeyer–Peppas

The underlined values indicate the highest coefficient of determination (R^2) obtained among the kinetic models evaluated for each formulation, and the corresponding model was considered the best fit for describing the drug-release kinetics.

Table 7: Stability study of formulation CF2 (1%, 2%, and 3%) and CF11 (1%, 2%, and 3%)

Day	Parameter	Formulation					
		CF2 (1%)	CF2 (2%)	CF2 (3%)	CF11 (1%)	CF11 (2%)	CF11 (3%)
Day 0	Physical appearance	milky White	White milky	milky White	White milky	milky White	White milky
	pH	6.62	6.63	6.50	6.45	6.78	6.22
	Viscosity (10rpm)	51300	31300	24400	52400	31900	23900
	% drug release (8h)	88.3	89.2	90.3	90.4	93.7	91.5
Day 30	Physical appearance	Milky white	White milky	Milky white	White milky	Milky white	White milky
	pH	6.61	6.61	6.52	6.46	6.76	6.21
	Viscosity	50280	31300	24400	52390	31900	23980
	% drug release (8h)	86.4	88.6	90.1	89.5	93.6	91.5
Day 60	Physical appearance	White milky	White milky	White milky	White milky	White milky	White milky
	pH	6.60	6.62	6.63	6.41	6.74	6.18
	Viscosity	50190	30900	22890	51730	31730	22503
	% drug release (8h)	84.7	88.1	89.4	87.5	92.7	89.6
Day 90	Physical appearance	Creamish	Light brown	White milky	Creamish	White milky	Creamish
	pH	5.80	6.61	6.45	6.43	6.73	6.20
	Viscosity	50100	27000	21090	51650	31690	22147
	% drug release (8h)	83.3	87.5	85.7	81.4	92.4	83.7

Burn induction

Full-thickness excision wounds (2.5 cm diameter) were created using a heated metal rod under ether anesthesia.[50]

Wound contraction measurement

Wound area was measured on days 3, 6, 9, and 12. Percentage contraction was calculated as[51]

$$\text{Percent wound contraction} = \frac{Healed\ area}{Total\ area} \times 100$$

RESULTS AND DISCUSSION

Optimization of cubosomes (BBD approach)

A total of 12 experimental runs were created using the BBD for optimization of cubosomal formulations.[52] The PS ranged from 112.4 to 381.2 nm, PDI from 0.171 to 0.537, and EE from 74.4% to 91.38%.

Response surface analysis confirmed that GMO concentration had the most important impact on PS and EE, whereas Poloxamer 407 showed a pronounced effect on PDI.[53]

EE

The EE ranged from 74.4% to 91.38%, indicating efficient encapsulation of the drug within the lipid bilayer structure of cubosomes.[54]

PS and zeta potential

The PS of formulation CF1-CF12 ranges from 112.4 to 381.2 nm, that confirm the formation of nanosize cubosomal dispersions.[53] Zeta potential of formulation CF1–CF12 ranged from −17.1 to −26.2 mV, which indicates the good colloidal stability due to sufficient electrostatic repulsion b/w particles.[55] This stability is important for preventing aggregation and maintaining long-term formulation integrity. The experimental results of the cubosomal formulation obtained using Box- Behnken design are presented in Table 2.

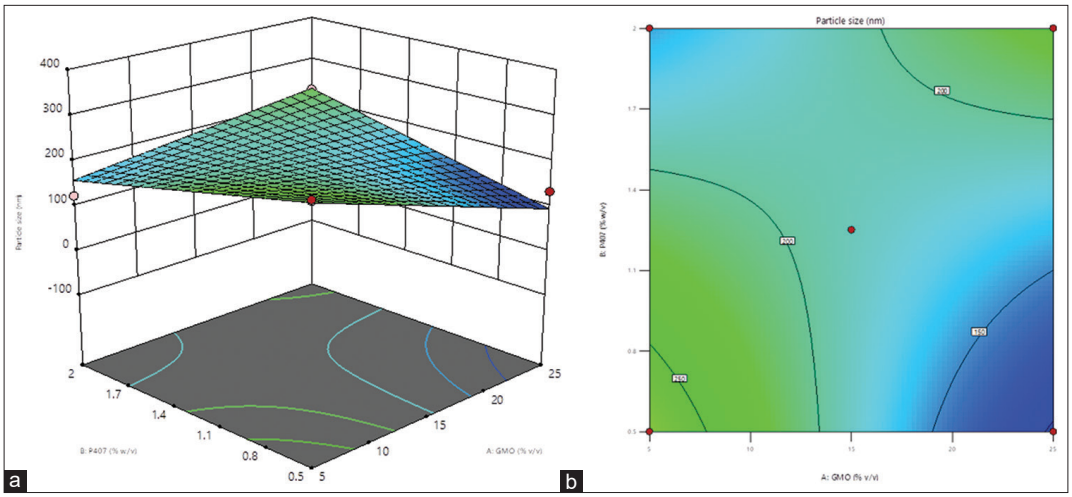

Figure 1: (a) Response surface 3D plot and (b) contour plot for particle size

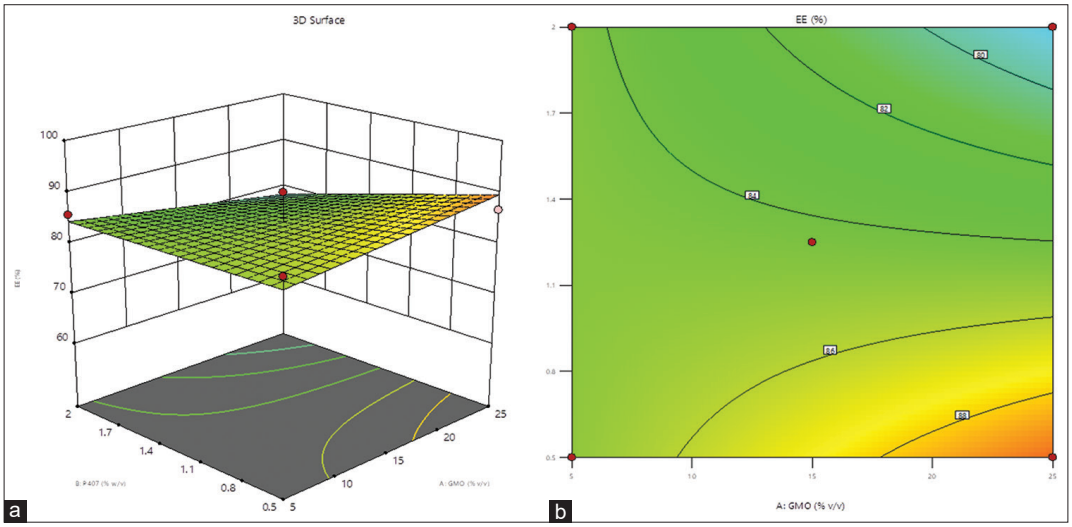

Figure 2: (a) Response surface 3D plot and (b) contour plot for entrapment efficiency

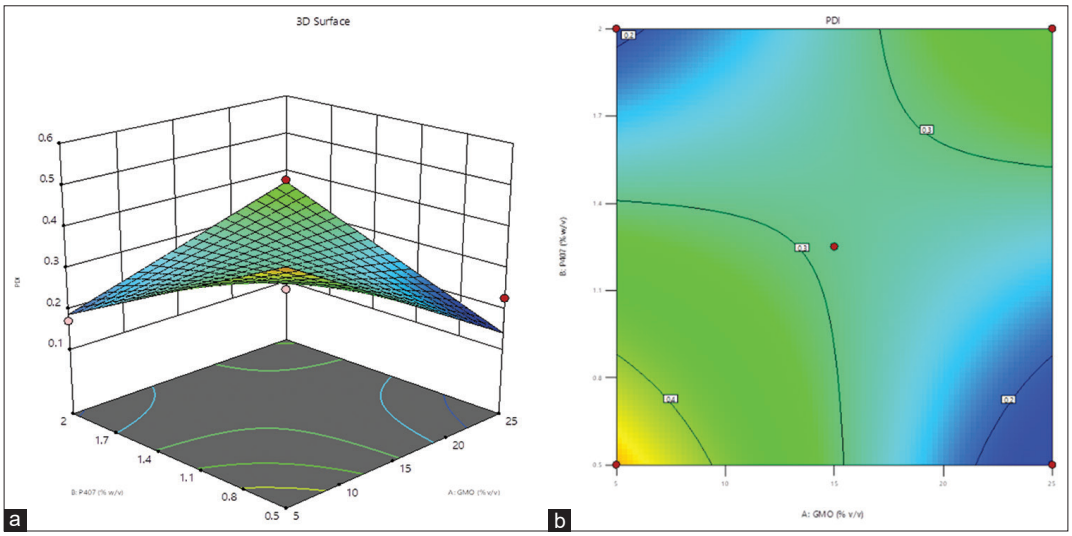

Figure 3: (a) Response surface 3D plot and (b) contour plot for polydispersity index

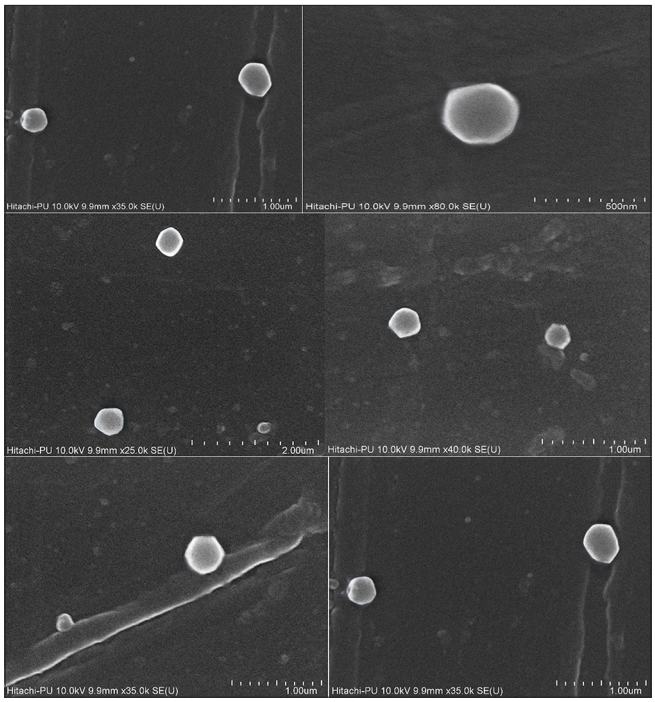

Figure 4: Field emission scanning electron microscopy image of different batches of cubosome dispersion

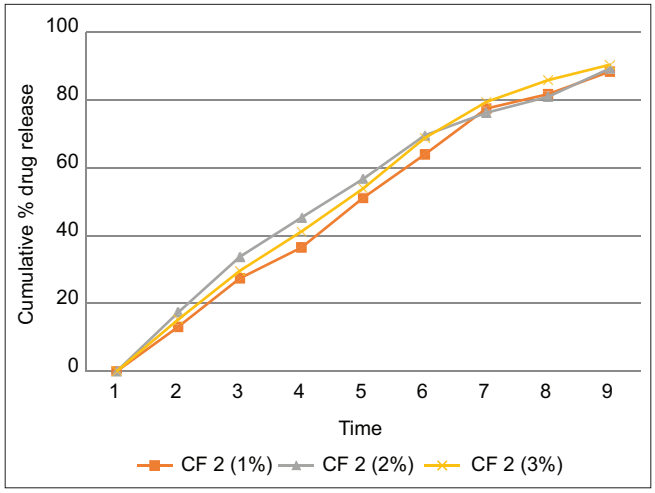

Figure 5: Graph of drug release of formulation CF2 (1%, 2% and 3%)

The three D response surface and contour plots illustrated the impact of constraints on the response variables, i.e., PS, EE, and PDI. All these plots are shown in Figures 1-3.

Morphology

FESEM analysis established well-defined cubic morphology with unchanging dispersion and minimal aggregation, validating successful cubosome formation.[56] FESEM images of the optimized cubosomal formulation are presented in Figure 4.

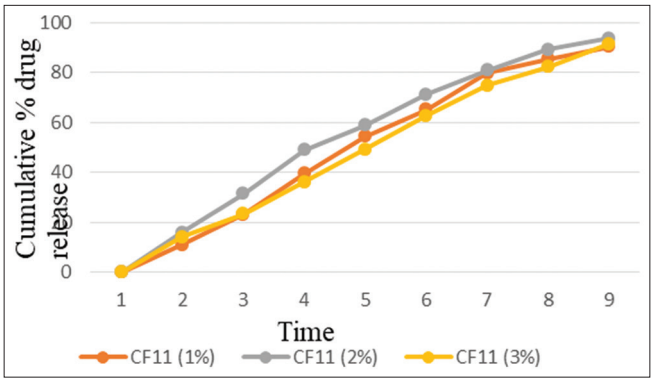

Figure 6: Graph of drug release of formulation CF11 (1%, 2% and 3%)

Table 8: Percentage wound contraction

Group	Day 0	Day 3	Day 6	Day 9	Day 12
I	-	14.86	19.00	27.73	34.21
II	-	10.90	15.24	21.38	28.30
III	-	20.84	24.70	26.58	34.77
IV	-	17.35	17.35	17.35	22.40

Cubosomal gel evaluation

All cubosomal formulations exhibited acceptable pH (6.2–6.8), suitable for topical application. Viscosity decreased with increasing shear rate, indicating pseudoplastic behavior. The pH values of prepared cubosomal gel formulation are presented in Table 3 and the viscosity of CF2 and CF11 formulation are presented in Table 4 and 5.

In vitro drug release

Both CF2 and CF11 formulations demonstrated a sustained release over 8 h with cumulative release exceeding 88–93%. The *in-vitro* drug release profile of the optimized cubosomal gel formulation are presented in Figure 5 and 6.

CF11 (2%) showed the most controlled and consistent release profile across all drugs.[57]

Kinetic modeling

SSD followed the Higuchi diffusion model (R^2 = 0.9966). Tretinoin and Vitamin D followed the Korsmeyer–Peppas model ($R^2 \approx 0.9992$). This indicates diffusion-controlled and anomalous transport mechanisms. The different models of drug release is shown in Table 6 and Figure 7.

Stability study

Formulations remained stable for 90 days with minor changes in pH, viscosity, and drug release. CF11 (2%)

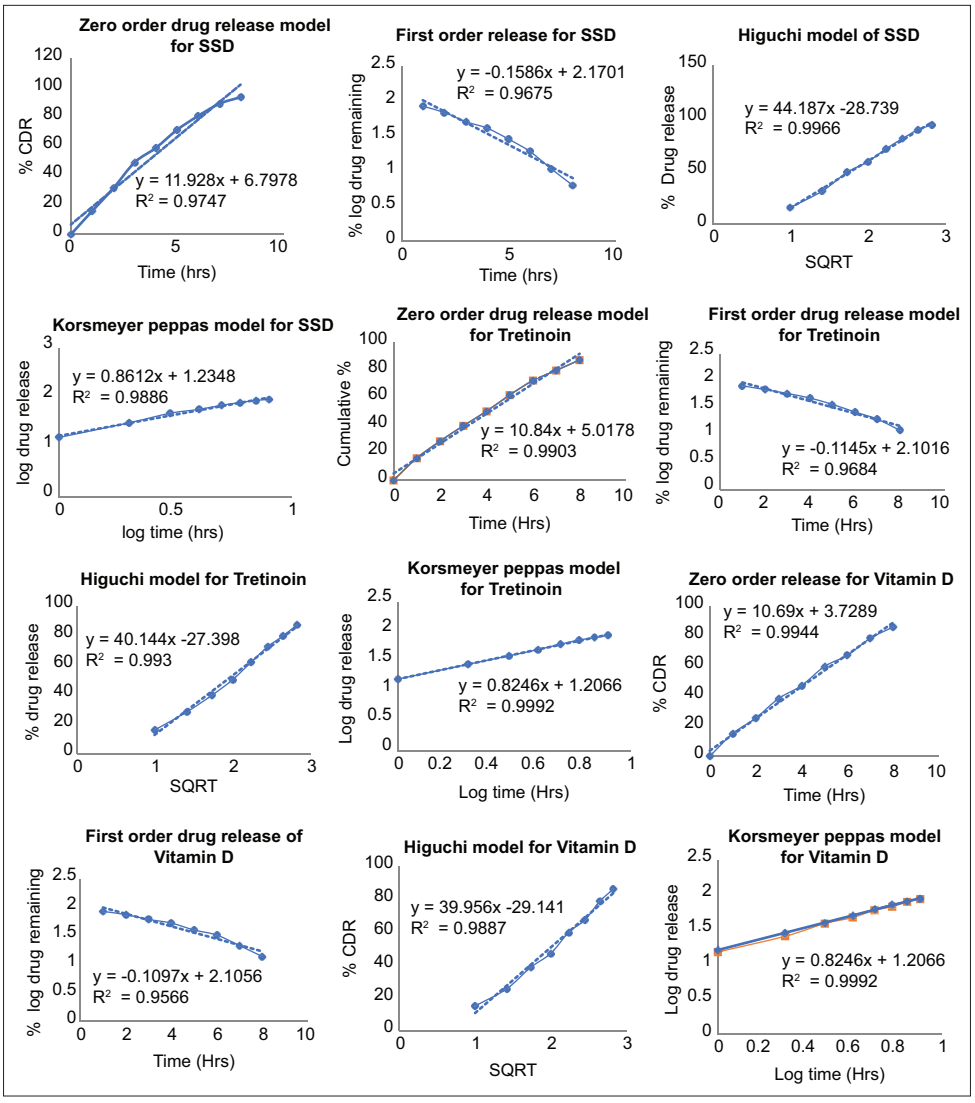

Figure 7: Different kinetic models for silver sulfadiazine, tretinoin and vitamin D

showed optimal stability shown in Table 7 and was selected for further studies.

In vivo wound healing

Group III (SSD + Tretinoin + Vitamin D cubosomes) showed the highest wound contraction (34.77% by Day 12), indicating synergistic healing activity. The control group exhibited the lowest healing response.

The percentage of wound contraction for each group at each time point (excluding Day 0) is shown in Table 8:

CONCLUSION

Cubosomal-based delivery systems successfully improved the encapsulation and sustained release of SSD, tretinoin, and Vitamin D. The optimized formulation (CF11 2%) confirmed superior physicochemical stability, sustained drug release, and enhanced wound healing efficacy, making it a promising topical delivery system for the management of burns.

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