https://doi.org/10.48047/AFJBS.6.1.2024.333-348

DESIGNING AND OPTIMIZATION OF CUBOSOME OF ISOLATED COMPOUND GINGEROL FROM ZINGIBER OFFICINALE RHIZOME

KARISHMA A NIKOSE[1]*, LAXMIKANT N BARDE[1]

School of Pharmacy, SunRise University, Alwar, Rajasthan, India.

Corresponding Author E-mail – chetanbarde21@gmail.com

Volume6,Issue1,2024

Received:15May2024

Accepted:10June2024

doi:10.48047/AFJBS.6. 1.

2024.333-348

ABSTRACT

This study focuses on the formulation and optimization of cubosomes encapsulating gingerol, a bioactive compound isolated from the rhizome of Zingiber officinale (ginger). Cubosomes, nano-structured lipid carriers, were designed to enhance the bioavailability and therapeutic efficacy of gingerol, which is known for its anti-inflammatory and antioxidant properties. The research employed various techniques for analyzing the entrapment efficiency, cubosome size, shape and morphology. The Cubosomal gel characterization was done by visual and physical parameters including homogeneity, viscosity, pH, spreadability. In vitro drug release parameters were also assessed. The optimized cubosomes exhibited enhanced encapsulation efficiency and controlled release, indicating their potential as an effective delivery system for gingerol. The findings underscore the promise of cubosome-based delivery in enhancing the medicinal benefits of herbal compounds.

Keywords: Cubosomes, Zingiber officinale, Encapsulation, Bioavailability

INTRODUCTION

Nature has all the answers for the many health problems and illnesses bothering this modernized and evolved society. Because of their numerous health benefits and little negative effects, nature has provided a variety of naturally occurring bioactive plants for the treatment of various ailments. These plants have been widely used for several centuries worldwide **(Atmakuri *et al.*, 2010)**. Plants have been used for nutrition and healing since prehistoric times **(Bhokare *et al.*, 2016).** The modern world has undoubtedly taken notice of the use of herbal medicine for basic healthcare in developing countries **(Goldberg, 1994).** Plant-based treatments have certain drawbacks, including low stability, low lipid solubility, and the need for a thoroughly tested procedure for ingredient separation and purification

(Kulkarni, 2011). In fact, it is the manufacturer's primary duty to get past these obstacles, give the product enough stability, and allow patients to consume it in a safer manner. In conventional medication, the substance has usually only reached a certain percentage of the target site. Because of the medicine's physicochemical characteristics, the majority of the dosage was distributed throughout the body, lowering its therapeutic potency **(Sharma *et al.*, 2017; Kumar and Rai, 2012)**. The realm of natural products has consistently provided a treasure trove of therapeutic compounds, with ginger (*Zingiber officinale*) standing out as a notable example due to its rich profile of bioactive constituents. Among these, gingerol, particularly [6]-gingerol, is distinguished for its significant pharmacological properties, including anti-inflammatory, antioxidant, and anticancer effects **(Ali *et al.*, 2008).** However, the bioavailability of gingerol poses a major challenge due to its poor water solubility and rapid metabolism.

To address these limitations, the formulation of cubosomes presents a promising approach. Cubosomes, also known as new colloidal nanodispersions, are stabilized by surfactants and feature a bicontinuous cubic phase in water **(Abdel-Bar *et al.*, 2017).** These nanoparticles, which can encapsulate both lipophilic and hydrophilic medicines, are created by emulsifying lipid phases, mostly GMO, in water **(Said *et al.*, 2021; Said *et al.*, 2018; Rarokar and Khedekar, 2018).** They are more stable than liposomes (Wakaskar, 2018), allow for longer drug release, and have a higher drug loading capacity because of their liquid crystalline structure **(Mathews *et al.*, 2022).** Additionally, they can efficiently transport proteins via their aqueous channels **(Almoshari, 2022)**. Compared to liposomes, they are less viscous and have a less hydrophobic core **(Zhang *et al.*, 2020)**; they can also shield the medication that is enclosed **(Ou *et al.*, 2018).** Cubosomes are nanostructured liquid crystalline particles that offer an advanced delivery system capable of enhancing the solubility, stability, and controlled release of lipophilic drugs like gingerol. These nano-vehicles, characterized by their cubic phase structures and high surface area, provide an effective means to improve the pharmacokinetic profile of bioactive compounds, thereby maximizing their therapeutic potential **(Eposito *et al.*, 2005).**The present study focuses on the design and optimization of cubosomes encapsulating the isolated compound gingerol from *Zingiber officinale* rhizome. The optimization process is crucial to achieving an ideal formulation that ensures maximum drug loading, stability, and sustained release. Various parameters, including the concentration of surfactants, stabilizers, and the method of preparation, are systematically evaluated to enhance the efficacy of the cubosome formulation.This research not only aims to overcome the limitations associated with the bioavailability of gingerol but also contributes to the

broader field of nanotechnology-based drug delivery systems. By improving the delivery and effectiveness of gingerol, this study opens new avenues for its application in treating various ailments, thereby underscoring the potential of integrating traditional medicinal compounds with modern pharmaceutical technologies.

2. MATERIAL AND METHODS

2.1 Method of preparation

2.1.1Preparation of gingerol cubosomes

The method used for the preparation of cubosomes was top-down method9. Varying concentrations of Glceryl Mono Oleate (GMO) along with Poloxamer 407 as shown in Table 1, were accurately weighed and heated on an electric water bath at a temperature of 40 to 45ºC until Poloxamer 407 completely dissolved in GMO. To the above solution drug was added and was mixed well. The clear lipid solution obtained was added slowly to distilled water and was subjected to probe sonication for 10 min. The resultant solution obtained was white opaque dispersion without presence of any aggregates. The prepared dispersions were stored in closed glass vials at room temperature for 72hrs, protected from light and later evaluation was carried out.

2.2 Preliminary optimization of process parameters

2.2.1 Optimization by Box Behnken Design (DOE)

For optimization of formulation Response Surface Methodology (RSM) used to study quantitative aspects of the impacts and interactions between key formulation factors of cubosomes. To inoculum the different process parameters at three levels (low, medium, and high, coded as -1, 0, and+1), a Box-Behnken Design with a total of 13 experimental runs was used. The Poloxamer 407: GMO ratio (A), lipid: isolated compound gingerol (B), and sonication duration (C) were used as independent factors, and their effects on size (Y1) and percentage entrapment (Y2) were evaluated as dependent variables. And the same parameter used for the preparation of the standard gingerol formulation. The Design-Expert programme (version 8.0.4, State-Ease Inc., Minneapolis, USA) was used for experiment design and analysis, as well as producing three-dimensional response surface and contour plots. The optimized batch was selected on the basis of desirability criteria. % prediction error of the prepared batch was calculated in order to evaluate the reliability of

developed mathematical models **(Sharma *et al.,* 2012)**

2.3 Characterization of cubosomes

2.3.1 Determination of particle Size (PS) and Zeta Potential (ζ)

Mean Particle size of isolated and standard gingerol loaded cubosomes was determined by using dynamic light scattering mehod (Zetasizer Nano ZS, Malvern, and Worcestershire, UK). The Zeta Potential of cubosomes were measured using the laser Doppler method. Each batch was analyzed in triplicate. For PS and ζ, analysis was carried out for 100 s and 60s resp. at room temperature by keeping angle of detection at 90˚.**(Gill *et. al.,* 2014)**

2.3.2 Entrapment Efficiency (EE)

The efficiency of gingerol (standard) and isolated compound entrapment in the cubosomes was determined by analyzing the amount of drug content of cubosomes in comparison to the total amount added. For this purpose, a pre-weighed quantity of the cubosomes were subjected to disruption using PBS (pH 7.4), and the amount of drug released drug was measured by UV .The following formula was used to calculate the entrapment efficiency**.(Ucisik, *et. al.,* 2015)**

Entrapment efficiency (%) = (Determined drug content) / (Total drug added) X100.

2.3.3 Shape and morphology

The shape and surface morphology of the prepared cubosomes was studied by both scanning electron microscopy (JEOL, JSM-6100).

2.4 Preparation of optimized cubosomal gel

The prepared nanodispersion of ketoconazole loaded cubosomes, was next added to Carbopol 934 aqueous dispersion and stirred at 350 RPM for uniform dispersion of the cubosomes in the aqueous system. Water was added to this system to make up the final weight of the formulation to 50 g. After uniform dispersion of cubosomes 30 µL of triethanolamine was added for neutralization of Carbopol to form gel.

2.4.1 Characterisation of the cubosomal gel

- **Visual characterisation**

The physical appearance or visual characterisation of the gel was analysed by the visually and normal human senses . The organoleptic properties included color , odor, and texture .

- **Physical characterisation**

- **Homogeneity:**

Physical appearance and homogeneity of the prepared gels were evaluated by visual perception.

- **Viscosity**

Viscosity of gel was determined using Brookfield viscometer (S-62, model LVDV-E) at 25 ^{o}C with a spindle (Spindle No.S64) speed of the viscometer rotated at 30 rpm.

- **pH**

pH measurement of the gel was carried out using a digital pH meter by dipping the glass electrode completely into the gel system to cover the electrode. The measurement was carried out in triplicate and the average of the three readings was recorded.

- **Spreadability**

Two glass slides of 20 cm × 20 cm were selected. The phytosomal gel was placed between the slides. A 100 g was placed on the upper slide to press the gel uniformly to format the inlayer. The time taken for the separation was noted using a top clock. The following equation was used for this purpose

$S = m \times L / T$

Where, S – Spreadability, m-Weight tied to the upper slide, l-Length of the glass t - Time taken in seconds.

2.5*In-vitro* drug release

Two checkpoint formulations, selected post numerical optimization and tallied by navigating through the design space, were prepared and subjected to in vitro drug release study using a dialysis membrane. Both the dispersions containing cubosomes equivalent to 5 gm of optimized gel formulation of containing standard and isolated gingerol were sampled out (3 mL) and inserted in a dialysis bag (12–14 kDa molecular weight cutoff; Himedia, India). The dialysis bag was immersed in 100 mL of release media (containing 90% pH 7.4 phosphate buffer saline and 10% methanol) under stirring at 400 RPM at 37 ± 0.5 °C. 3 mL of aliquots were withdrawn at predetermined time intervals and the same volume was replenished by fresh release media. Samples withdrawn were analyzed by UV spectrophotometer and the absorbance obtained was then used to calculate the amount of drug release at given time intervals. Various release models including Zero order, First order, Higuchi, and Korsmeyer-Peppas models were utilized to determine the drug release behavior from the designed formulations. The following mathematical equations of various release models were used:

Zero order model: $\mathbf{Q = k^0 t + Q^0}$

First order model:$Q = Q^0ek1t$

Higuchi model:Q = kHt1/2

Korsmeyer Peppas model: $Q = kKPt^n$

Where, Q and Q0 represent the amount of ketoconazole released at the time-point, t and 0, respectively; $k^{0,}$ k^1, kH, and kKP represent rate constants ketoconazole releasing in zero-order, first-order, Higuchi and Korsmeyer-Peppas models, respectively. Besides these, n corresponds to the release exponent implying the mechanism of releasing.

If n = 0.5 or n < 0.5, the drug release mechanism is followed by Fickian diffusion. If 0.5 < n < 1.0, it is non-Fickian or anomalous diffusion mechanism. If n = 1.0, the mechanism is non-Fickian case II diffusion. In case of n > 1.0, non-Fickian super-case II diffusion mechanism is followed.

2.6 Storage Stability Studies

The developed cubosomes stored at temperature 25 ± 2°C and 60% RH and 40 ± 2°C and 75% RH for a period of 90 days as per the ICH guidelines. If Instabilities occur in cubosomes formulations caused by hydrolysis or oxidation of the molecules and are indicated by leakage of the encapsulated drug and alteration in vesicle size due to fusion and aggregation. These formulations were examined by any alteration in particle size and residual drug content at regular intervals, i.e., 30, 60 and 90 days.(**Gupta *et al.*, 2007**).

3. RESULT AND DISCUSSION

3.1Optimization of formulation via DOE

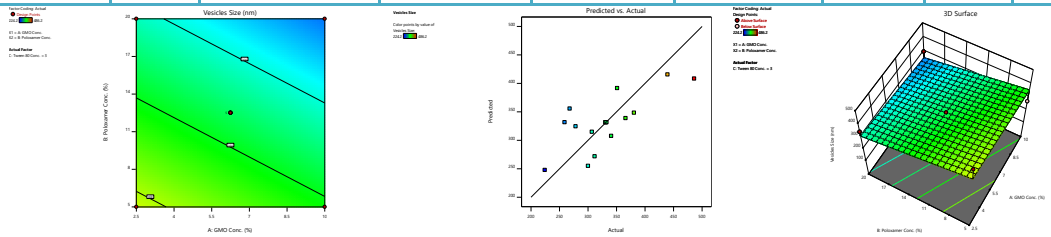
Design of Expert software version 11.2.2 - Box-Behnken Designs, STAT 503 used for the optimization of the formulation. Below tables shown the result and optimization of formulation as per Box-Behnken Designs through response surface methodology , after the selection of optimized formulation, isolated compound stigmasterol and standard stigmasterol actively loaded in the formulation.

Table 1: Different formulation independent variables

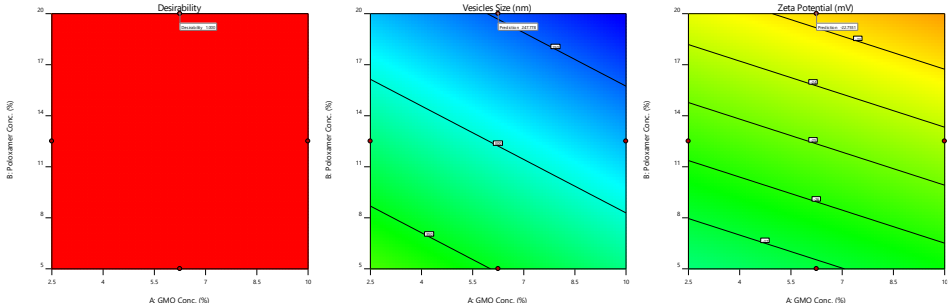
Factor	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A (x1)	GMO Conc.	%	Numeric	2.50	10.00	-1 ↔ 2.50	+1 ↔ 10.00	6.25	2.65
B(x2)	Poloxamer Conc.	%	Numeric	5.00	20.00	-1 ↔ 5.00	+1 ↔ 20.00	12.50	5.30
C(x3)	Tween 80 Conc.	%	Numeric	1.0000	5.00	-1 ↔ 1.00	+1 ↔ 5.00	3.00	1.41

Table 2: Different formulation dependent variables

Response	Name	Units	Observations	Analysis	Minimum	Maximum	Mean	Std. Dev.	Ratio	Transform	Model
R1 (y1)	Vesicles Size	nm	17	Polynomial	224.2	486.2	331.65	63.89	2.17	None	Linear



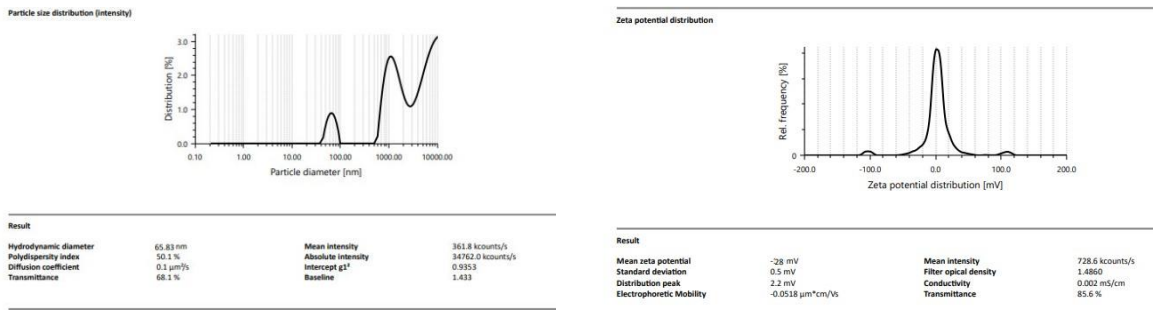
Graph 1: Response surface plot showing combined effect of Entrapment efficiency of formulations.



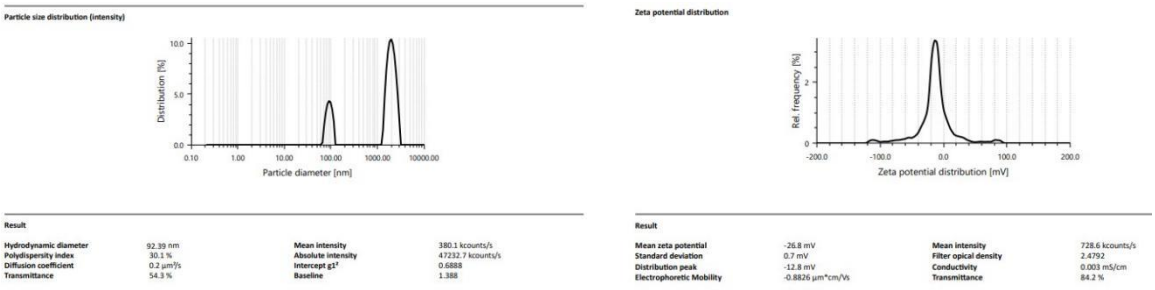
Graph 2: A shows the overlay graph of independent variable, B shows the response surface graph of independent variables and C shows the response graph of the zeta potential

3.2 Characterization of cubosomes (optimization formulation)

3.2.1 Zeta Potential (ζ) and Particle sizer (PS) of optimized formulation



Graph 3: Particle size and Zeta potential of the optimised formula of isolated compound gingerol



Graph 4: Particle size and Zeta potential of the optimised formula of standard compound gingerol

Table 3: Particle sizerand zeta potential study of the isolated and standard gingerol

Formulation	Formulation code	Vesicles size	PDI	Zeta potential

Cubosomes Formulation containing isolated compound gingerol	**CBIC**	92.39	30.1%	-28 mv
Cubosomes Formulation containing standard compound gingerol	**CBSC**	65.83	50.1%	-26.8 mv

Zetasizer (Particle sizer) Nano ZS (Malvern Instruments Ltd, UK) determined the diameter often distinct formulations. Size distribution curve indicated a variationin particle size in a range of 75–550 nm. Similarly, the diameter of isolated compound gingerol loaded cubosomes was found as 92..39 nm , with an average PDI value of 30% and the diameter of standard gingerol loaded cubosomes was found as 65.83 nm, with an average PDI value of 50.1%. The zeta potential of the isolated and standrad gingerol was --28 mV and -26.8 mV.

3.2.2 Entrapment Efficiency (EE) of the optimized cubosomes formulation

The EE of the isolated compound loaded cubosomes and standard gingerol loaded cubosomes were shown in the below table: through the result it was found that isolated compound gingerol shows the equivalent entrapment efficiency like the standard gingerol.

Table 4: Entrapment efficiency of formulation

Formulation	Formulation code	Entrapment efficiency %
Cubosomes Formulation containing isolated compound gingerol	**CBIC**	**79.6±0.22**
Cubosomes Formulation containing standard stigma stertol	**CBSC**	**84.2±0.21**

3.2.3 Shape and morphology

The morphological study of the optimized formulation of cubosomes loaded isolated compound gingerol and standard gingerol were performed by the SEM analysis (JOEL) and result shown the spherical shape of cubosomes with different sizes and result shown in the table and **figure 1**.

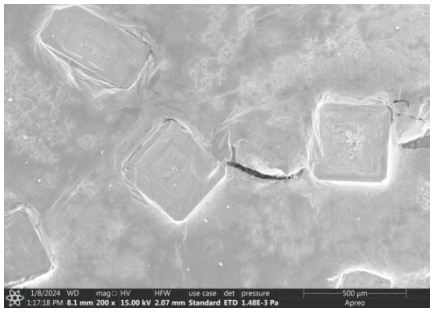


Figure 1: showing the SEM image of the isolated compound gingerol loaded cubosomes

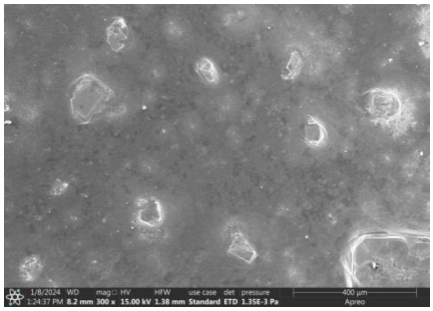


Figure 2: showing the SEM image of the standard gingerol loaded cubosomes

Table 5: SEM analysis of the optimized formulation

S.NO.	Formulation code	SEM analysis
1	CBIC	NMT = 330 nn
2	CBSC	NMT = 280 nn

Note: NMT : not more than

3.3 Optimised Cubosomal gel Preparation

3.3.1 Preparation of gel

The prepared nanodispersion of isolated gingerol and standard gingerol loaded cubosomes was formulated by Direct Dispersion method. Cubosomal gel was obtained by adding a weighed amount of carbopol 934 (2% w/w) to distilled water and kept for half a day, not forgetting the swelling of carbopol, and then triethanolamine was added to adjust the pH. The resulting gel was then diluted with an appropriate amount of cubosomal dispersion at a 1:1 (w/w) ratio of dispersion to gel.

Table 6: Constituent of preparation of gel

S.N	CONSTITUENTS	CBIC GEL	CBSC GEL
1	Carbopol 934	2	2
2	Propylene glycol	1 ml	1ml
3	Triethanolamine	0.5 ml	0.5 ml
4	Gingerol std	200 mcg	200 mcg
5	Gingerol isolated	200 mcg	200mcg
6	Distilled water	Up to 50ml	Up to 50ml

3.3.2 Characterisation of gel

- **Visual characterisation**

On careful visual inspection against dark and white background, all the prepared dermal gel formulations were found to be free from any suspended particulate matter. All the formulations were found to be translucent gel. The texture was non greasy and smooth in touch.

Table 7: represent the physical properties of the gel loaded with drug

S.N.	Formulation code	Parameters	Result
1	CBIC gel	Color	Light yellow in color
		Texture	Smooth
		Appearance	Pleasant
		Odour	Characteristic
		Gritty	Non gritty
S.N.	**Formulation code**	**Parameters**	**Result**

1	CBSC gel	Color	Yellowish in color
		Texture	Smooth
		Appearance	Pleasant
		Odour	Characteristics
		Gritty	Non gritty

- **Physical characterisation**
- **Homogeneity:**

The homogeneity of the cubosomal gel of the optimised formulation were show that the formulation PUFG12 was properly homogenised mixture. This uniformity was required for the proper penetration of the drug.

Table 8*:* represent the homogeneity of the cubosomal gel loaded with drug

S.N.	Formulation code	Parameters	Result
1	CBIC gel	Homogeneity	Homogenous mixture
2	CBSC gel	Homogeneity	Homogenous mixture

- **Viscosity**

The viscosity of the optimised formulation were reported in the cp (unit) . after the measurement of the viscosity it was found that the PUFG12 was slightly viscous at 30 rpm with spindle no. 64

Table 9: represent the Viscosity of the cubosoamal gel loaded with drug

S.N.	Formulation code	Parameters	Result
1	CBIC gel	Viscosity	2449±0.054
2	CBSC gel	(Viscosity (cps) at Room Temperature ± SD.)	2745±0.044

- **pH**

The pH of the optimised formulations from was found to be in the range of 6 to 7 ideally, the dermal gel should possess pH in the range of 6-7, so as to minimize discomfort or irritation due to acidic pH and microbial growth due to basic pH..

Table10*:* represent the pH of the phytosomal gel loaded with drug

S.N.	Formulation code	Parameters	Result
1	CBIC gel	pH.	7.1±0.011
2	CBSC gel		7.3±0.052

- **Spreadability**

The Spreadability of the formulations is a characteristic derived from its more basic property i.e. viscosity. The greater the viscosity the longer will be the time taken for spreading. The spread- ability of the optimised formulations was easily spreadable.

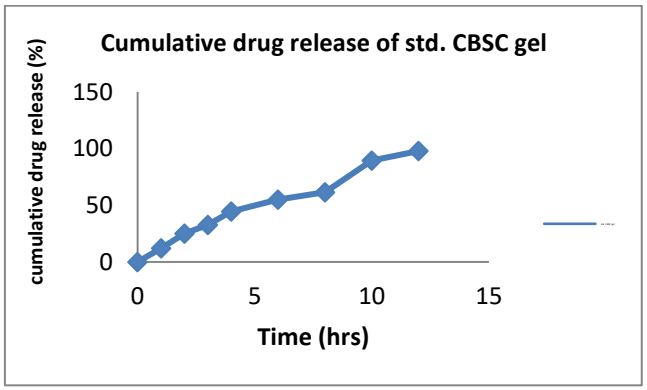
Table 11: represent the spreadability of the cubosomal gel loaded with drug

S.N.	Formulation code	Parameters	Result
1	CBIC gel	Spreadability	29.3 ±0.065
2	CBSC gel	(gm.cm/ sec)	28.15±0.042

3.4 In-vitro drug release of optimized formulation cubosomes loaded isolated compound gingerol and standard gingerol.

a) *In-vitro* drug release of optimized formulation cubosomes loaded standard gingerol

The *In-vitro* drug release of the cubosomes formulation containing standard gingerol shows 97.82 % active release at 12 hrs and its kinetic release study graph of zero order , first order , Higuchi and Korsmeyer peppas shows the regression in the below table.



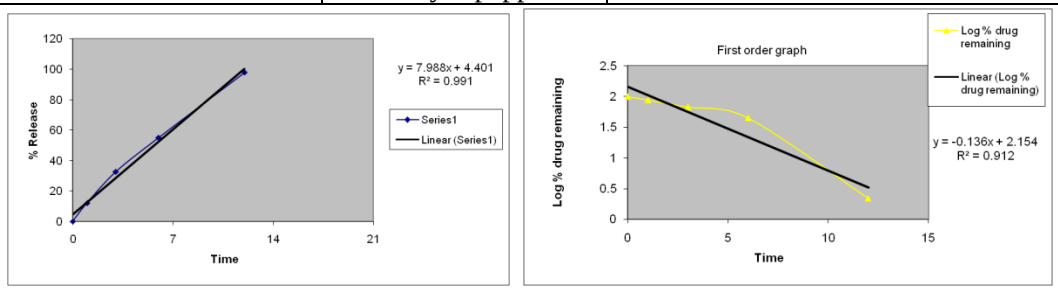
Graph5: Cumulative drug release of std. gingerol CBSC gel

Table 12: Release kinetics study of standard gingerol

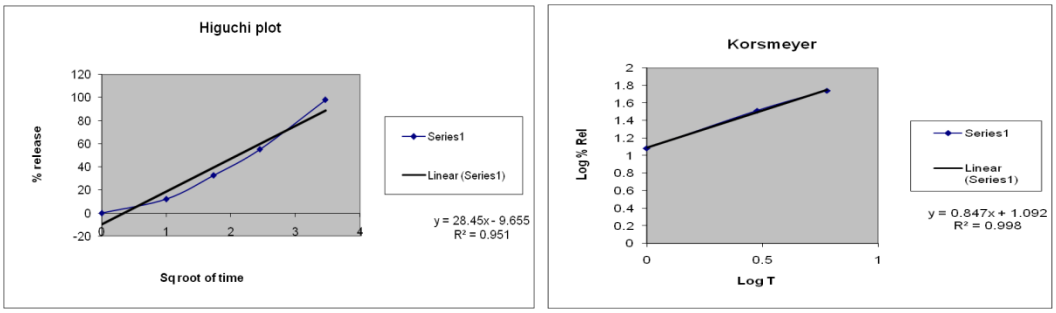
S. No	Time (hrs)	cumulative % drug released	% drug remaining	log Cumu % drug remaining	Square root time	log time	Log % Rel	CDR % drug remaining
1.	0	0	100	2	0	0	0	4.641589
2.	1	12.18	87.82	1.943593	1	0	1.085647	4.444925
3.	2	25.09	74.91	1.87454	1.414214	0.30103	1.399501	4.215476
4.	3	32.63	67.37	1.828467	1.732051	0.477121	1.513617	4.069011
5.	4	44.61	55.39	1.743431	2	0.60206	1.649432	3.81192
6.	6	55.13	44.87	1.651956	2.44949	0.778151	1.741388	3.553465
7.	8	61.56	38.44	1.584783	2.828427	0.90309	1.789299	3.374902
8.	10	89.45	10.55	1.023252	3.162278	1	1.95158	2.19323
9.	12	97.82	2.18	0.338456	3.464102	1.079181	1.990428	1.296638

Table 13: Correlation value (R^2 value)

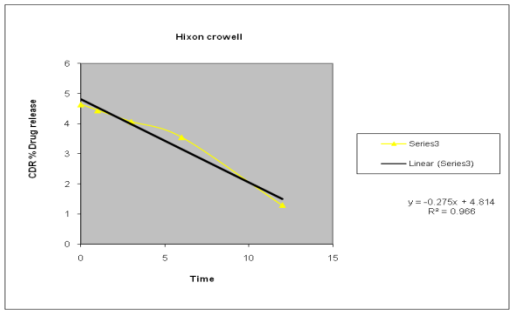
Formulation	Model	Kinetic parameter values
CBIC gel	Zero Order	0.991
	First Order	0.912
	Higuchi	0.951
	Hixon crowell	0.966
	Korsmeyer peppas	0.998



Graph 6: Zero order kinetic model and First Order kinetic model



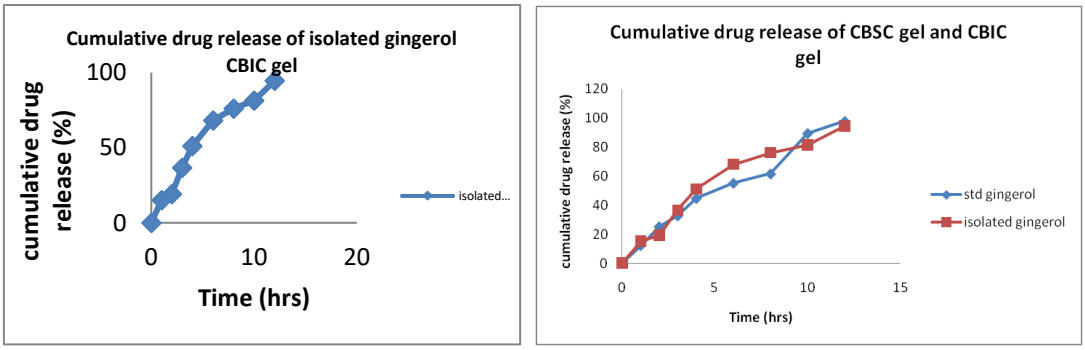
Graph 7: Higuchi model and Korsmeyer peppas



Graph 1: Hixon crowell

3.4.1 *In-vitro* drug release of optimized formulation cubosomes loaded Isolated compound gingerol

The *In-vitro* drug release of the cubosomes formulation containing isolated gingerol shows 94.51 % active release at 12 hrs and its kinetic release study graph of zero order , first order , Higuchi and Korsmeyer peppas shows in the below regression table.



Graph 9: Cumulative drug release of isolated gingerol CBIC gel and Cumulative drug release of isolated gingerol CBIC and CBSC gel

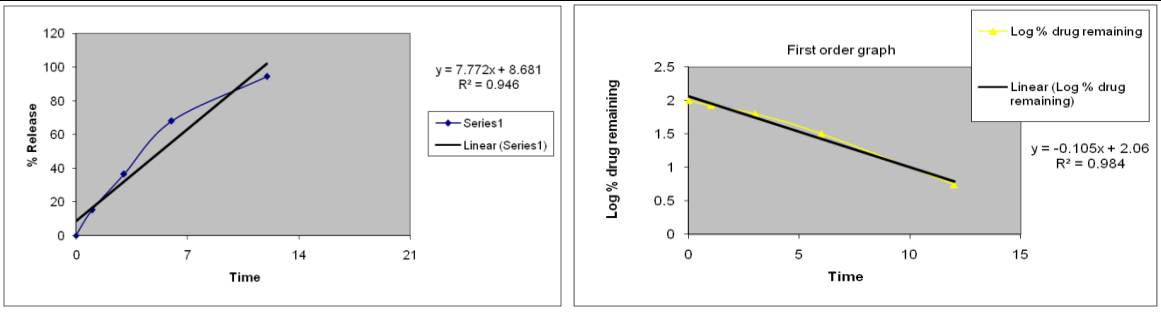
Table 11: Release kinetics study of Isolated compound gingerol

S. No	Time (hrs)	cumulative % drug released	% drug remaining	log Cumu % drug	Square root time remaining	log time	Log % Release	CDR % drug remaining
1.	0	0	100	2	0			4.641589
2.	1	15.21	84.79	1.928345	1	0	1.182129	4.393206
3.	2	19.16	80.84	1.907626	1.414214	0.30103	1.282396	4.323898
4.	3	36.56	63.44	1.802363	1.732051	0.477121	1.563006	3.988299
5.	4	51.12	48.88	1.689131	2	0.60206	1.708591	3.656316
6.	6	68.11	31.89	1.503655	2.44949	0.778151	1.833211	31.89
7.	8	76.02	23.98	1.379849	2.828427	0.90309	1.880928	23.98

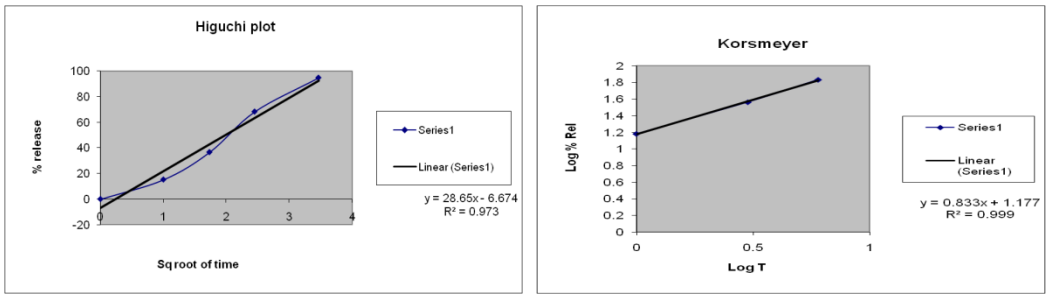
8.	10	81.45	18.55	1.268344	3.162278	1	1.910891	18.55
9.	12	94.51	5.49	0.739572	3.464102	1.079181	1.975478	5.49

Table 12 : Correlation value (R^2 value)

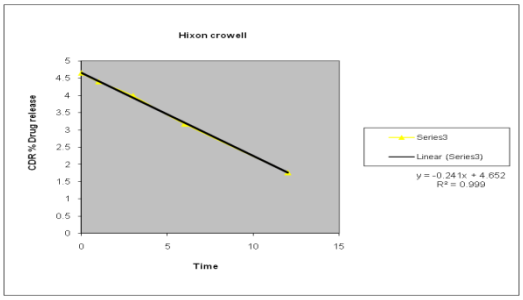
Formulation	Model	Kinetic parameter values
CBSC Gel	Zero Order	0.946
	First Order	0.984
	Higuchi	0.973
	Hixon Crowell	0.999
	Korsmeyer peppas	0.999



Graph 20: Zero order kinetic model and First Order kinetic model



Graph 3: Higuchi model and Korsmeyer peppas



Graph 4: **Hixon crowell**

3.5 Storage Stability Studies

The optimized formulation CBIC and CBSC gel were placed in the stability test chamber and subjected to stability studies at accelerated testing (25^0C ± 2^0C and 60 ± 5% RH) and (40^0C ± 2^0C and 70 ± 5% RH) for 3 months. The formulation were checked for parameter like Particle size and entrapment efficiency at the interval of 30, 45, 60, 90 days (3 month) months. The formulation was tested for stability under accelerated storage condition for 3 months in accordance to International Conference on Harmonization (ICH) guidelines. Results of assay and evaluation at periodic time points of stability studies are summarized in

below Table. 52 .All Results were compared against final formulation of 0 days as the reference.

Table 16: Stability Study of optimized cubosomes formulation containing isolated compound gingerol

S.No	Time (Days)	25^0C±2 ^{0}C and 60 ± 5% RH			
		Particle size of isolated gingerol	entrapment efficiency of isolated gingerol	Particle size of standard gingerol	entrapment efficiency of standard gingerol
1.	0	331.1	73	278.6	74
2.	30	330.9	73	288.1	74
4.	60	330.5	73	237.5	74
5.	90	330.1	72.9	236.2	73.9

Table 17 : Stability Study of optimized cubosomes formulation containing standard gingerol

S.No	Time (Days)	40^0C ± 2 ^{0}C and 70 ±5% RH			
		Particle size of isolated gingerol	entrapment efficiency of isolated gingerol	Particle size of standard gingerol	entrapment efficiency of standard gingerol
1.	0	330.1	73	278.6	74
2.	30	320.1	73	218.3	74
4.	60	329.6	72.9	227.5	73.9
5.	90	319.3	72.8	215.1	73.8

The selected optimized formulations were evaluated for stability studies which were stored at temperature of 25° ± 2°C and 60%± 5% RH and 40^0C ± 2 ^{0}C and 70 ±5% RH for 90 days (3 month) and were analyzed for their particle size (nm) and entrapment efficiency . There was no significant change in the properties of optimized cubosomes formulation during the stability period. There was a slight decrease in particle size for the stored formulation, but it was well within the acceptable limit. Results of assay and evaluation at periodic time points of stability studies were summarized in above Table.

4. CONCLUSION

In this study, we successfully designed and optimized cubosomes incorporating the bioactive compound gingerol, isolated from *Zingiber officinale* rhizome. The formulated cubosomes exhibited enhanced stability, controlled release, and improved bioavailability of gingerol. This innovative approach underscores the potential of cubosome carriers in enhancing the therapeutic efficacy of phytoconstituents like gingerol, paving the way for advanced drug delivery systems in natural product-based therapeutics.

5. REFERENCES

- Abdel-Bar, H. M., & Abd el Basset Sanad, R. (2017). Endocytic pathways of optimized resveratrol cubosomes capturing into human hepatoma cells. Biomedicine & pharmacotherapy, 93, 561-569.
- Ali, B. H., Blunden, G., Tanira, M. O., & Nemmar, A. (2008). Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): a review of recent research. Food and chemical Toxicology, 46(2), 409-420.

- Almoshari, Y. (2022). Development, therapeutic evaluation and theranostic applications of cubosomes on cancers: An updated review. Pharmaceutics, 14(3), 600.
- Atmakuri, L. R., & Dathi, S. (2010). Current trends in herbal medicines. J Pharm Res, 3(1), 109-113.
- Bhokare, S. G., Dongaonkar, C. C., Lahane, S. V., Salunke, P. B., Sawale, V. S., & Thombare, M. S. (2016). Herbal novel drug delivery: A review. World Journal of Pharmacy and Pharmaceutical Sciences, 5(8), 593-611.
- Esposito, E., Cortesi, R., Drechsler, M., Paccamiccio, L., Mariani, P., Contado, C., ... & Puglia, C. (2005). Cubosome dispersions as delivery systems for percutaneous administration of indomethacin. Pharmaceutical research, 22, 2163-2173.
- Goldberg, B. (1994). Alternative medicine: The definitive guide future medicine. Washington: Puyallup, 257.
- Kulkarni, G. T. (2011). Herbal drug delivery systems: An emerging area in herbal drug research. Journal of Chronotherapy and Drug Delivery, 2(3), 113-119.
- Kumar, K., & Rai, A. K. (2012). Miraculous therapeutic effects of herbal drugs using novel drug delivery systems. International Research Journal of Pharmacy, 3(2), 27-30.
- Mathews, P. D., Mertins, O., Angelov, B., & Angelova, A. (2022). Cubosomal lipid nanoassemblies with pH-sensitive shells created by biopolymer complexes: A synchrotron SAXS study. Journal of Colloid and Interface Science, 607, 440-450.
- Ou, N., Sun, Y., Zhou, S., Gu, P., Liu, Z., Bo, R., ... & Wang, D. (2018). Evaluation of optimum conditions for Achyranthes bidentata polysaccharides encapsulated in cubosomes and immunological activity in vitro. International journal of biological macromolecules, 109, 748-760.
- Rarokar, N. R., & Khedekar, P. B. (2018). Cubosomes: a vehicle for delivery of various therapeutic agents. MOJ Toxicol, 4(1), 19-21.
- Said, M., Aboelwafa, A. A., Elshafeey, A. H., & Elsayed, I. (2021). Central composite optimization of ocular mucoadhesive cubosomes for enhanced bioavailability and controlled delivery of voriconazole. Journal of drug delivery science and technology, 61, 102075.
- Said, M., Elsayed, I., Aboelwafa, A. A., & Elshafeey, A. H. (2018). A novel concept of overcoming the skin barrier using augmented liquid nanocrystals: Box-Behnken optimization, ex vivo and in vivo evaluation. Colloids and Surfaces B: Biointerfaces, 170, 258-265.

- Sharma, R., Hazra, J., & Prajapati, P. K. (2017). Nanophytomedicines: A novel approach to improve drug delivery and pharmacokinetics of herbal medicine. Bio Bull, 3(1), 132-5.
- Wakaskar, R. R. (2018). General overview of lipid–polymer hybrid nanoparticles, dendrimers, micelles, liposomes, spongosomes and cubosomes. Journal of drug targeting, 26(4), 311-318.
- Zhang, L., Li, J., Tian, D., Sun, L., Wang, X., & Tian, M. (2020). Theranostic combinatorial drug-loaded coated cubosomes for enhanced targeting and efficacy against cancer cells. Cell death & disease, 11(1), 1.