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Research

Development and Evaluation of Lycopene and Alpha-Tocopherol-Loaded Ethosomal Gel Optimized by Design of Experiments for Managing Oral Sub-Mucosal Fibrosis

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ABSTRACT:

Background: Oral sub-mucosal fibrosis (OSMF) is a chronic fibro-inflammatory disorder marked by progressive fibrosis leading to collagen deposition in the epithelial layer of the mouth. Trismus and dysfunction may result from an effect on the underlying masticatory muscles. Ethosomes were fabricated using a cold method. **Aim:** This research focused on the formulation of a novel ethosomal gel encapsulating lycopene, which exhibits both antioxidant and anti-inflammatory properties, and Alpha-Tocopherol, a well-known antioxidant, aimed at enhancing localized therapy for OSMF. Ethosomes are systematically optimized through Design of Experiments (DOE) to fine-tune vesicle size, drug loading, and release behaviour. **Result:** The optimized ethosomal gel exhibited a pH of 6.5 ± 0.01, good viscosity, and spreadability (1.76 ± 0.03 g·cm/s). It achieved entrapment efficiencies of 85.24% for lycopene and 83.67% for alpha-tocopherol. It provided sustained drug release, indicating suitability for controlled buccal delivery. Cumulative drug release reached 45.53%, 63.03%, and 85.72% (Lycopene) and 21.74%, 42.95%, and 77.13% (Alpha-Tocopherol) at 1, 5, and 24 h, confirming sustained delivery and good vesicle stability.

Keywords: Ethosomal gel, Lycopene, Alpha-Tocopherol, Oral Submucosal Fibrosis

INTRODUCTION

Oral submucosal fibrosis is a chronic condition characterized by stiffening and thickening of the oral submucosa, resulting in limited mouth mobility and discomfort.[1] The disease is primarily associated with areca nut chewing, which induces excessive collagen synthesis and oxidative damage.[2] This process typically includes the formation of reactive oxygen species (ROS), stimulation of transcription factors such as nuclear factor-kappa B (NF-κB), and increased expression of pro-inflammatory cytokines like tumour necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β).[3]

Currently available therapies broadly address symptom control rather than the reversal of

fibrosis. Lycopene and Alpha-Tocopherol possess antioxidant properties that may help alleviate oxidative stress and fibrosis.[4] The anti-inflammatory property of Lycopene inhibits the transforming growth factor.[5] Ethosomes, lipid vesicles enriched with ethanol, offer enhanced permeation through mucosal tissues by disrupting lipid barriers.[6]

MATERIALS AND METHODS

Materials

The key materials included Lycopene, Alpha-Tocopherol (Alkem Laboratories), phospholipids (Soya Lecithin), Chloroform, Ethanol, Tween 80, and Carbopol polymer, procured from established suppliers.

Formulation Optimization Using DoE

A three-factor, three-level Box–Behnken design evaluated the influence of soya lecithin (X_1), Tween-80 (X_2), and ethanol (X_3) on vesicle size, PDI, zeta potential, and entrapment efficiency.[7,8] After adding the variables, it gave 17 runs.

Based on the results of the 17 formulations, again BBD gave 19 solutions from which I have chosen 1 solution as the optimized formulation.

Preparation of Ethosomes

Ethosomal vesicles were prepared using the cold method. Soya lecithin, together with Lycopene and Alpha-Tocopherol, was dissolved in ethanol with continuous constant stirring by using a magnetic stirrer at 1200 rpm, then heated the phase at 30 ^{0}C. The aqueous phase was heated at the same temperature added gradually, producing flexible vesicles capable of encapsulating the drugs.[9,10] After the sonication process was done for 5 minutes to reduce vesicle size.

Characterization of Ethosomes

Incompatibility studies: FTIR studies were done after the lyophilization process of the optimized formulation to check the interaction between the drug and excipients.[11]

Particle size & PDI: By using the standard method, I have taken a 100 µl sample of formulation and mixed it with the HPLC water in the cuvette, then fixed the cuvette in Dynamic Light Scattering (Horiba SZ-100) and noted the reading.[12]

Zeta potential: Evaluated to predict the stability of the vesicle.[13]

Entrapment efficiency: Entrapment efficacy was determined via ultracentrifugation at 12,000 rpm for 1 hour, followed by spectrophotometric drug estimation.[14]

%Entrapment efficiency = Amount of drug in sediment x100\Total amount of drug

In-vitro drug release: Done by using dialysis against 0.1 M SDS/NaCl medium due to the solubility of both drugs at 37 °C, samples were withdrawn up to 24 h.[15,16]

Gel formulation:

Accurately weigh Carbopol 934 was add it to pure water, keeping it in the refrigerator for hydration for 2-3 hours. After hydration, 10ml of the optimized ethosomes were dispersed in gel (1%) neutralized with triethanolamine.[17,18]

Characterization of Gel formulation:

pH test of gel:

Calibrated the pH meter at 25 °C. Disperse 1.0 g of the formulated ethosomal gel in 10 mL of purified water, equilibrate for 10 minutes, and measure the pH by dipping the pH meter electrode into the beaker.[19]

Viscosity:

Viscosity was determined by using the Brookfield viscometer.[20] Place the
cleaned beaker on the viscometer platform. Firstly, pour an appropriate amount of gel (30–50 mL), depending on the beaker. into the beaker. Remove surface bubbles with a spatula. RV-4 spindle is used at 20 RPM for 30-50 sec. Wait till the stability of the reading.[21]

Spreadability:

The parallel plate method is used. The spreadability of the ethosomal gel was determined by placing 1.0 g of gel at the centre of a glass slide and carefully placing another slide of the same dimensions over it. Then, a weight of 50 g was applied centrally and left for a minute to ensure uniform spreading. After 1 minute, the outline of the spread gel was traced and transferred to graph paper; the spread area (cm^2) was then calculated.[22,23]

Then, Spreadability was calculated using the formula:

$$S=M\times L/T$$

Where:

- S = Spreadability (g·cm/s)
- M = Weight applied (g)

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Page No.: 25-33

- L = Distance moved by the upper plate (cm)
- T= = Time taken (s)

***In-Vitro* release study by using goat skin**

The in vitro drug release of optimised ethosomes was evaluated using goat mucosal skin. The goat mucosal skin was thoroughly washed with saline water at room temperature to eliminate any surface impurities. Subsequently, 2 mL of the ethosomal formulation was carefully introduced into the mucosal skin, and both ends were securely sealed with appropriate closures. The prepared assembly was then immersed in 100 mL of release medium, consisting of 0.1 M SDS and 0.1 M NaCl at a concentration of 1 mg/mL⁻, in a beaker. The release experiment was conducted at 37 °C under continuous stirring at 100 rpm using a magnetic stirrer. At predetermined time intervals (30 min, 1, 2, 3, 5, and 24 hours), 3 mL of the release medium was withdrawn. To maintain sink conditions, an equal volume of fresh dissolution medium was replenished immediately after each sampling. The collected samples were analysed using a UV spectrophotometer, by which the absorbance was measured to determine the drug concentration

RESULTS AND DISCUSSION

- **Fourier Transform Infra-Red Spectroscopy (FTIR) Study**

Fig 1:FTIR Spectrum of Lycopene

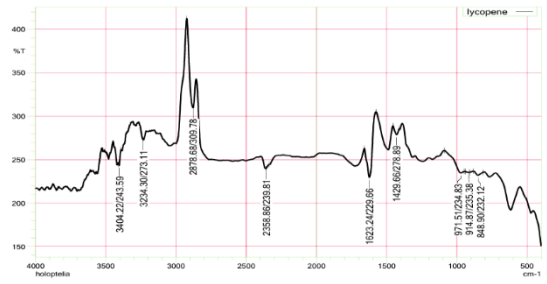


Fig 2:FTIR Spectru Alpha-Tocopherol

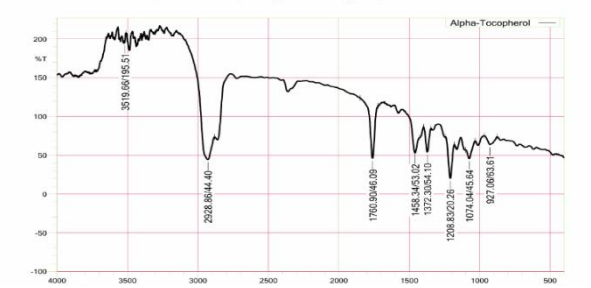


Fig 3:FTIR Spectrum of Placebo

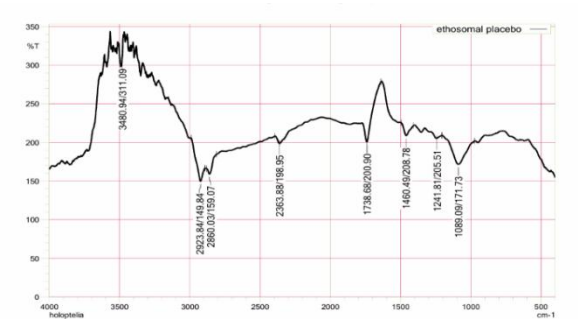
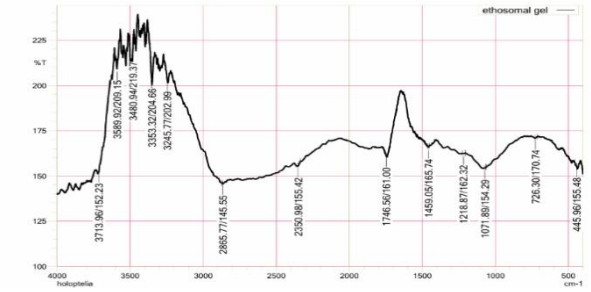


Fig 4:FTIR Spectrum of Ethosomal Gel



- **Particle Size:** As the amount of Tween 80 increased, particle size decreased.

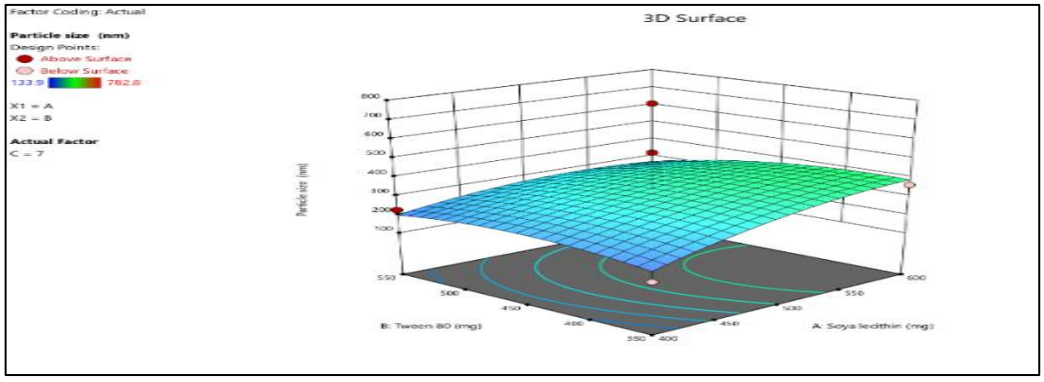


Fig 5: 3D Surface response plot of particle size

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Page No.: 25-33

- **Polydispersity Index (PDI):** In contrast, intermediate concentrations of both excipients produce more favorable PDI values, reflecting improved homogeneity.

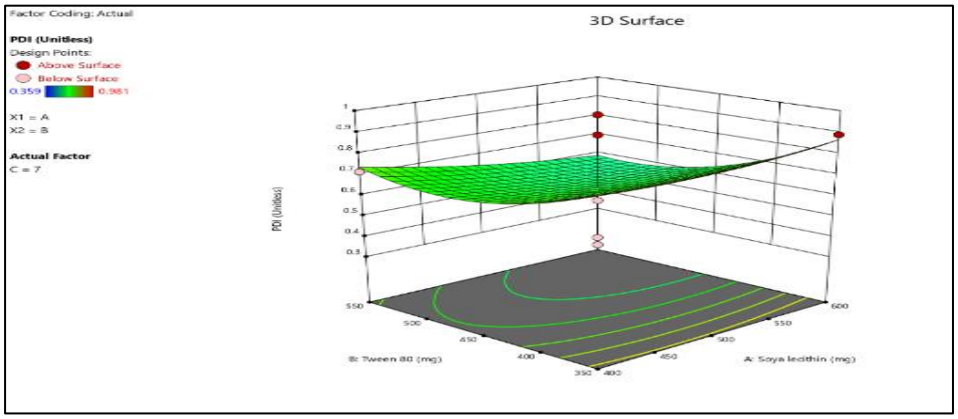

Fig 6: 3D Surface response plot of P.I

- **Zeta potential:** In this study, the zeta potential for Lycopene and Alpha-Tocopherol loaded ethosomal gel ranged from -40 to 30mV.

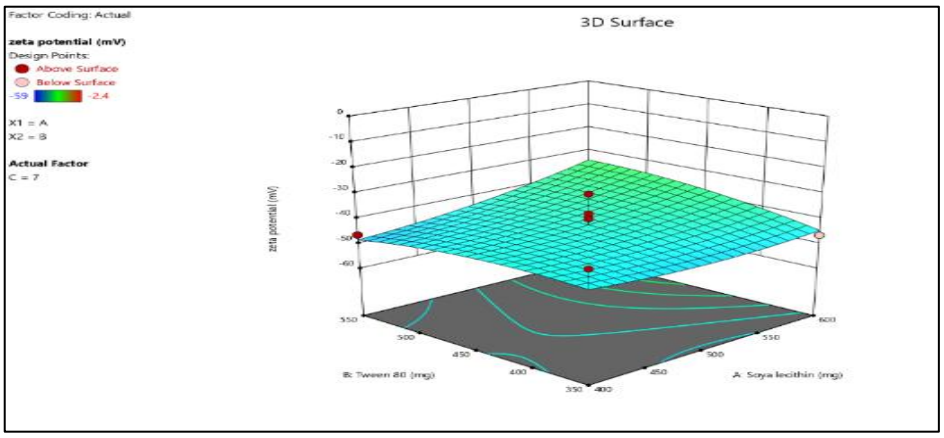

Fig 7: 3D Surface response plot of Zeta potential

- **%Entrapment Efficiency of Lycopene :** Entrapment efficiency increased as particle size decreased.

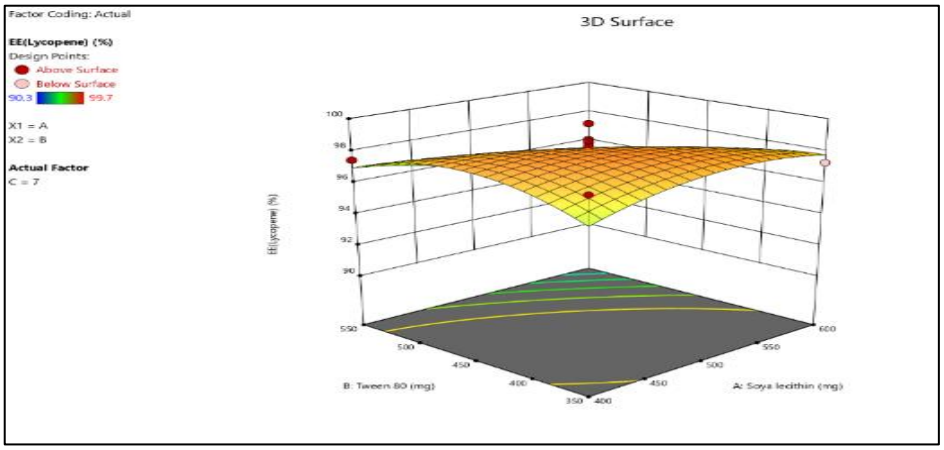

Fig 8: 3D Surface response plot of Entrapment Efficiency

- **%Entrapment Efficiency of Alpha-Tocopherol:** Entrapment efficiency increased as particle size decreased.

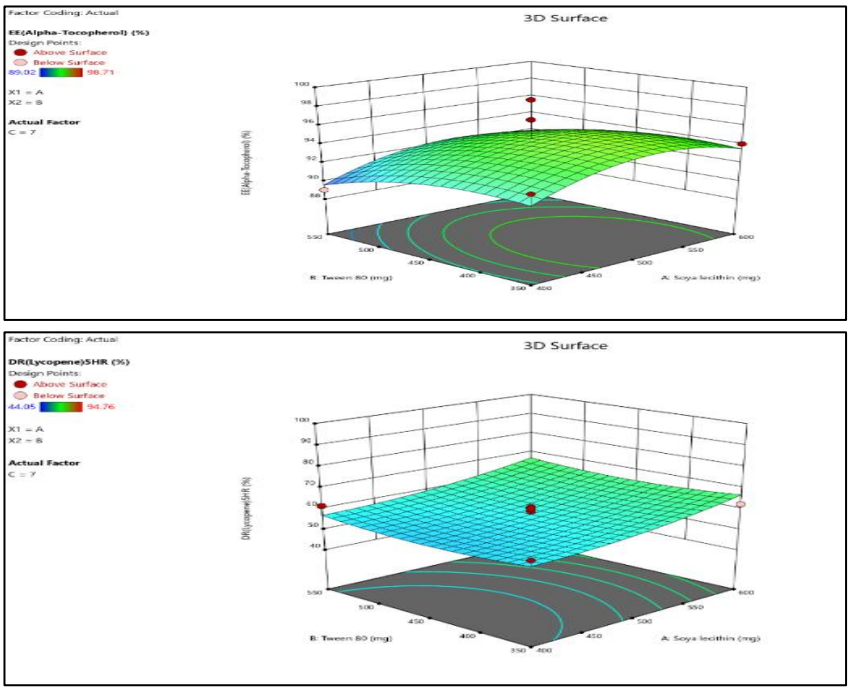

Fig 9: 3D Surface response plot of Entrapment Efficiency

- **Cumulative Drug Release of Lycopene at 1 Hour:** the drug release of lycopene after 1 hour increases with higher concentrations of soya lecithin (A) and Tween 80 (B).

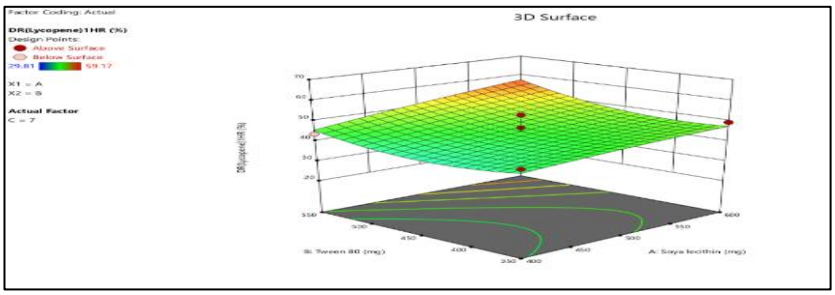

Fig 10: 3D Surface response plot of Cumulative Drug Release of Lycopene at 1 Hour

- **Cumulative Drug Release of Alpha-Tocopherol at 1 Hour:** Moderate to higher levels of soya lecithin and Tween 80 show slightly better release, while very low concentrations of both result in minimal release.

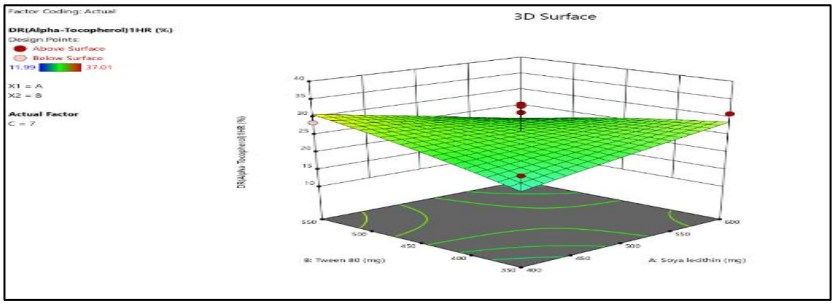

Fig 11: 3D Surface response plot of Cumulative Drug Release of Alpha-Tocopherol at 1 Hour

- **Cumulative Drug Release of Lycopene at 5 Hours:** The release ranges from approximately 44% at lower levels to nearly 95% at the upper range, with the best performance observed at high values of both factors.

Fig 12: 3D Surface response plot of Cumulative Drug Release of Lycopene at 5 hours

- **Cumulative Drug Release of Alpha-Tocopherol at 5 Hours:** Release is higher at moderate to high concentrations of soya lecithin and Tween 80, with the maximum observed toward the upper levels of both factors.

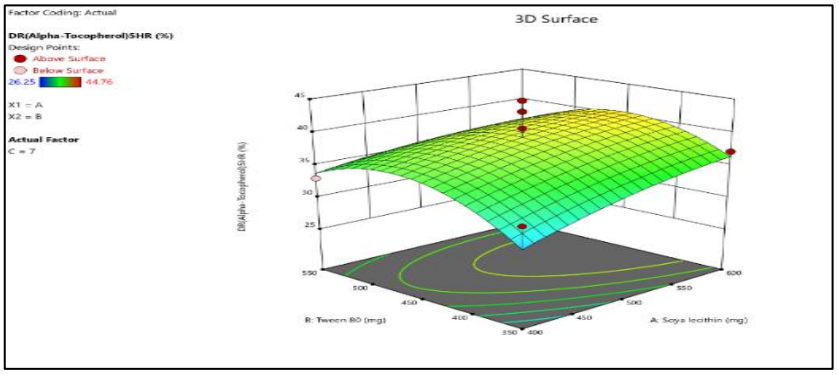

Fig 13: 3D Surface response plot of Cumulative Drug Release of Alpha-Tocopherol at 5 hours

- **Cumulative Drug Release of Lycopene at 24 Hours:** Optimal drug release was observed at moderate levels of lecithin and Tween 80.

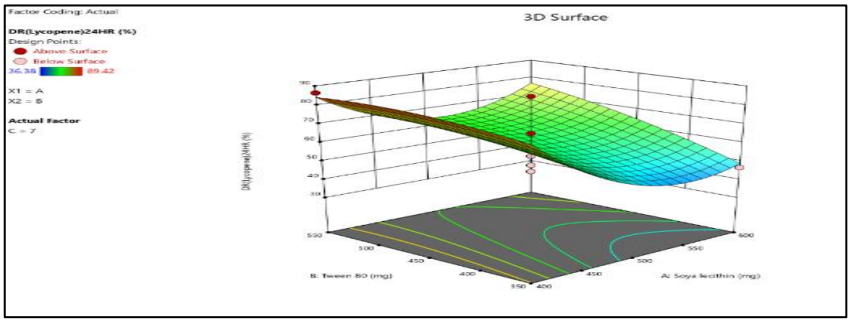

Fig 14: 3D Surface response plot of Cumulative Drug Release of Lycopene at 24 hours

- **Cumulative Drug Release of Alpha-Tocopherol at 24 Hours:** The graph shows that at lower levels of both lecithin and Tween 80, the drug release remains minimal. As the concentrations increase, release values improve.

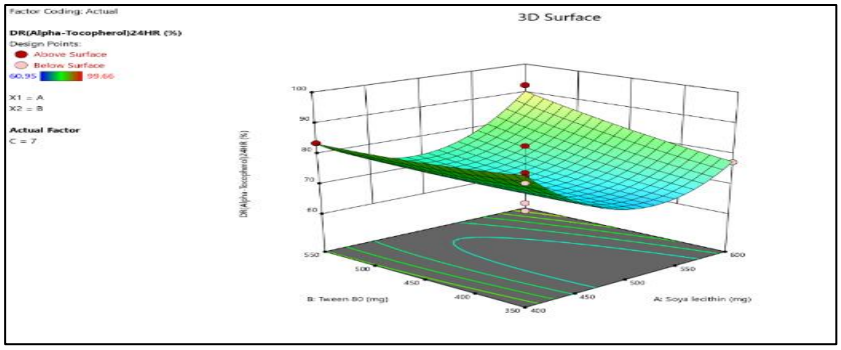

Fig 15: 3D Surface response plot of Cumulative Drug Release of Alpha-Tocopherol at 24 Hours

International Journal of Pharmaceutical Drug Design (IJPDD)
Website: https://ijpdd.org/
ISSN: 2584-2897
Vol. 2, Issue 11, November, 2025
Page No.: 25-33

Table 1 Optimized ethosomal characteristics:

Parameter	Optimized value (Mean ± SD, n = 3)
Particle size (nm)	127.16 ± 3
Polydispersity index (PDI)	0.45 ± 0.02
Zeta potential (mV)	–45 ± 2
Entrapment efficiency – Lycopene (%)	97.7 ± 2
Entrapment efficiency – α-Tocopherol (%)	98.76 ± 3

- **In-vitro release:**

Fig. 16: Drug Release of Lycopene of All the Formulations using BBD

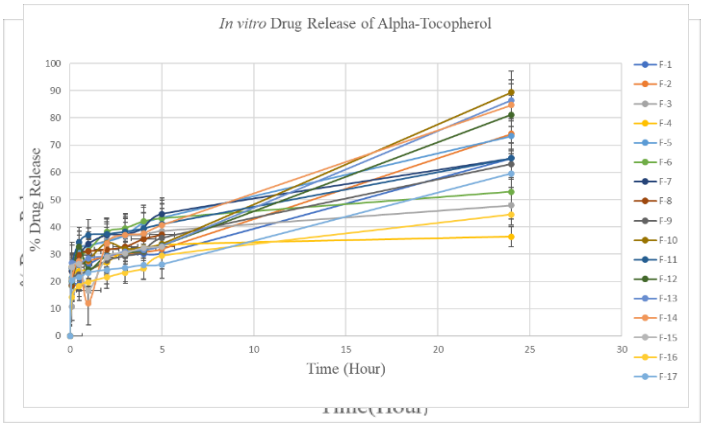

Fig. 17: Drug Release of Alpha-Tocopherol of All the Formulations using BBD

- **pH of Gel:** The pH of the evaluated Ethosomal gel was found to be 6.5.
- **The viscosity of the Gel:** The viscosity of the Ethosomal gel was measured using a Brookfield viscometer with a spindle number 7.

Table 2: Viscosity measured using Spindle No. 7

RPM	Viscosity	% Torque
20	188200 Centipoise	94%

- **Spreadability of the Gel:** Spreadability of the evaluated Ethosomal gel was found to be 1.7 g.cm/sec
- ***In-Vitro* release study by using goat skin:**

Table 3: % Drug Release of Lycopene and Alpha-Tocopherol

S. No	Time	% Drug release of Lycopene	% Drug release of Alpha-Tocopherol
1	0	8.36	21.36
2	0.1	10.11	27.58
3	0.5	16.36	31.14
4	1	19.24	36.48
5	2	20.62	44.49
6	3	20.99	51.16
7	4	22.24	60.95
8	5	23.12	64.51
9	24	49.52	76.52

CONCLUSION

This study developed an ethosomal gel encapsulating Lycopene and Alpha-Tocopherol, optimized by using DoE. In this work, the Quality by Design (QbD) strategy is utilized to systematically design, optimize, and assess ethosomal formulations to overcome solubility and stability-related challenges. Particle size values ranged from 133.9 to 782.8 nm, influenced mainly by soya lecithin concentration.High lecithin and ethanol tended to increase vesicle size (e.g., 782.8 nm at Std 6).

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The study concludes that the ethosomal gel system containing Lycopene and Alpha-Tocopherol Acetate is a promising localized treatment option for OSMF. The gel enhances patient compliance, with possible clinical benefits including improved mouth opening and reduction in burning sensation. Thus, the developed ethosomal gel provides a novel, safe, and efficient drug delivery approach that may significantly contribute to the management of OSMF and has potential applications for other oral mucosal pathologies as well.

Conflict of Interest

There is no conflict of interest regarding the data and publication of this manuscript.

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