International Journal of Pharmaceutical Drug Design (IJPDD)
Website: https://ijpdd.org/
ISSN: 2584-2897
Vol. 2, Issue 11, November, 2025
Page No.: 16-24

Research

Design Of Experiment Approach for Optimization and Characterization of Lycopene and Coenzyme Q10 Microemulsion for Treatment of Oral Submucosa Fibrosis

Harsh Pratap Singh Rajpoot*[1], Dr Aniruddha Pare[2], Shubham Pare[3], Bhumika Sahu[4]
[1,2,4]VNS group of institutions, Vidya Vihar, Neelbud, Bhopal – 462044, Madhya Pradesh, India
[3]Rosan Pharma Ltd., Indore

Corresponding Author:
Mr. Harsh Pratap Singh Rajpoot

Email:
h.p.423355@gmail.com

DOI: *10.62896/ijpdd.2.11.03*

Conflict of interest: *NIL*

Article History
Received: 12/10/2025
Accepted: 05/11/2025
Published: 28/10/2025

Abstract:
Background: Oral Submucous Fibrosis (OSMF) is a chronic, progressive disorder of the oral mucosa characterized by fibrosis, trismus, and burning sensation. Current therapies offer limited benefits due to poor solubility, stability, and bioavailability of drugs. Lycopene and Coenzyme Q10, with strong antioxidant and anti-fibrotic properties, have therapeutic potential but are limited by these formulation challenges. **Aim:** The study aimed to design, optimize, and characterize a microemulsion of Lycopene and Coenzyme Q10 using a Quality by Design (QbD)-based Design of Experiments (DoE) approach for effective management of OSMF. **Result:** A microemulsion was formulated using olive oil, Tween 80, and glycerol, optimized through a Box–Behnken Design. The optimized formulation showed a particle size of 52 nm, zeta potential of –31.9 mV, and a PDI of 0.366, indicating nanoscale uniformity and stability. Entrapment efficiency was 74.5% for Lycopene and 97.5% for Coenzyme Q10. In-vitro release studies demonstrated biphasic, sustained drug release, ensuring both immediate and prolonged antioxidant effects.

Keywords: Oral Submucous Fibrosis, Microemulsion, Lycopene, Coenzyme Q10, Design of Experiments, Antioxidants.

1. INTRODUCTION

Oral Submucous Fibrosis (OSMF) is a chronic, progressive disorder of the oral mucosa, strongly associated with areca nut chewing and tobacco use. It is characterized by excessive collagen deposition, stiffness of the oral mucosa, trismus, and burning sensation, with a significant risk of malignant transformation.[1] Globally, OSMF prevalence has exceeded 2.5 million cases, with India among the most affected regions.[2] Conventional treatments, including corticosteroids, enzymatic therapy, antioxidants, and surgical interventions, provide limited therapeutic success due to poor solubility, low bioavailability, and inadequate mucosal penetration of drugs.[3]

Lycopene and Coenzyme Q10 (CoQ10) are potent antioxidants with anti-inflammatory and anti-fibrotic effects, yet their application is restricted by poor aqueous solubility and stability.[4,5]

Microemulsions, due to their nanoscale droplet size, high solubilizing capacity, and improved mucosal penetration, represent a promising delivery system for such drugs. .[6]

The present study aims to design and optimize a Lycopene and CoQ10-loaded microemulsion using a Quality by Design (QbD) approach to improve solubility, stability, and site-specific delivery for effective management of OSMF.

2. MATERIALS AND METHODS

Materials The key materials used in this study included Lycopene and Coenzyme Q10 as the active pharmaceutical ingredients, Olive oil as the oil phase, Tween 80 as the surfactant, and Glycerol as the co-surfactant. All materials were procured from established suppliers provided by VNS Institute,

Bhopal. Lycopene and Coenzyme Q10 were selected for their potent antioxidant properties relevant to the management of oral submucous fibrosis. Olive oil was used to enhance the solubility and bioavailability of the lipophilic actives, while Tween 80 and Glycerol facilitated the formation and stabilization of the microemulsion system. All chemicals and reagents were of analytical grade and used without further purification.

2.1 Formulation Optimization Using DoE

A three-factor, three-level Box–Behnken design (BBD) was employed to evaluate the influence of Olive oil (X_1), Tween 80 (X_2), and Glycerol (X_3) on key microemulsion characteristics, including particle size, polydispersity index (PDI), zeta potential, and entrapment efficiency. Seventeen experimental runs were generated based on the selected variables. Analysis of these runs produced nineteen potential solutions, from which a single formulation exhibiting the most desirable characteristics was selected as the optimized microemulsion.[7]

Table 1: Formulation Protocol Using BBD

Formulation	Factor 1 A: Olive oil[g]	Factor 2 B: Tween 80[g]	Factor 3 C: Glycerol[g]
F1	1.35	6.5	2.125
F2	1.35	6.75	2
F3	1.35	6.25	2
F4	1.7	6.5	2
F5	1.7	6.5	2.25
F6	1.35	6.25	2.25
F7	1.35	6.5	2.125
F8	1.35	6.5	2.125
F9	1.35	6.5	2.125
F10	1.35	6.5	2.125
F11	1	6.5	2
F12	1	6.25	2.125
F13	1	6.75	2.125
F14	1.35	6.75	2.25
F15	1.7	6.75	2.125
F16	1.7	6.75	2.125
F17	1	6.5	2.25

2.2 Preparation of Microemulsion

An accurately weighed amount of Olive oil was placed in a beaker and covered with brown paper to protect Lycopene from photodegradation. Lycopene (100 mg) and Coenzyme Q10 (10 mg) were added to the oil phase along with a single drop of alpha-tocopherol as an antioxidant. The mixture was stirred continuously on a magnetic stirrer until both active ingredients were completely dissolved. Thereafter, the surfactant blend of Tween 80 and Glycerol was incorporated, and stirring was continued to ensure uniform mixing. Finally, distilled water was added Dropwise until the total volume reached 25 mL. After that, it was sonicated for 5 minutes, resulting in a stable and homogeneous microemulsion suitable for further evaluation.

2.3 Characterization of Microemulsion

Incompatibility studies:

FTIR studies were done after the lyophilization process of the optimized formulation to check the interaction between the drug and excipients.[8]

Physical stability:

The transparency of the microemulsion was assessed visually. Formulations were stored at 25°C for one week and monitored for precipitation, creaming, or cracking. Samples were also centrifuged at 10,000 rpm for 30 minutes to check for phase separation.[9]

Particle Size and Polydispersity Index (PDI):

Particle size and PDI were measured using a Horiba nanoparticle size analyzer via dynamic light scattering (DLS). Samples were diluted with HPLC-grade water.[10]

Zeta potential:

Zeta potential was determined using the same analyzer after diluting the samples with HPLC water. Measurements were performed at 25°C. [11]

Entrapment: Entrapment efficacy was determined via ultracentrifugation at 120,00 rpm for 1 hour, followed by spectrophotometric drug estimation.[12]

In Vitro Drug Release

Drug release from the optimized microemulsion was studied using the dialysis bag method. Dialysis membranes were soaked overnight in distilled water, and 2.5 mL of microemulsion was placed inside each bag. Bags were immersed in 100 mL of PBS (pH 7.4) containing 0.1% Tween 80 at 37°C with stirring at 100 rpm. Samples (3 mL) were withdrawn at predetermined intervals up to 24 hours and replaced with fresh medium. Drug concentration was measured by UV spectrophotometry [13]

2.4 Preparation of Microemulsion-Loaded Gel

The optimized Lycopene and CoQ10 microemulsion was incorporated into a gel using Carbopol 934 (100 mg) as the gelling agent. Carbopol was dispersed in 1 mL of distilled water using a mechanical stirrer (Remi, Mumbai, India) at 1200 rpm.

The drug-loaded microemulsion (10 mL) was then added to the gel base and stirred for 15 minutes to ensure uniform mixing. Triethanolamine (50 µL) was added to neutralize the dispersion, and the gel was allowed to stand overnight for complete swelling, resulting in a homogenous microemulsion-loaded gel suitable for topical application.

2.5 Characterization of ME Gel formulation:

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

Viscosity:

To determine the rheological properties, the formulated gel was placed in a beaker beneath the spindle (RV-7) and rotated at room temperature (25–27 °C) (20 rpm) in a Brookfield viscometer.

Spreadability

A 1 g sample of gel was placed between two glass slides, and a 50 g weight was applied for 20 seconds. The spread diameter was traced and transferred onto graph paper to calculate the spread area (cm²), indicating the ease of gel application.

In-Vitro Release Study by using goat mouth skin:

2.5 g of microemulsion gel was placed on the goat buccal mucosa, sealed, and immersed in 100 mL PBS (pH 7.4) with 0.1% Tween 80 at 37 °C. The system was stirred at 100 rpm and 32 ± 0.5 °C. Samples (3 mL) were withdrawn at predetermined intervals up to 24 h, replaced with fresh medium, and analyzed by UV spectrophotometry for drug concentration.

3. RESULTS AND DISCUSSION

Fourier Transform Infra-Red Spectroscopy (FTIR) Study

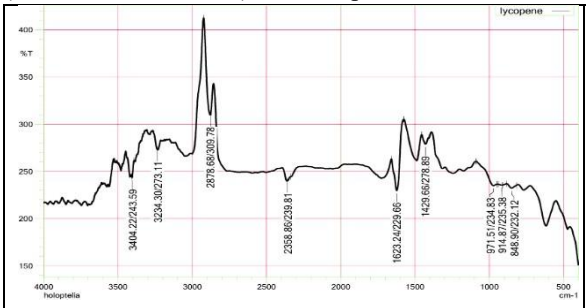

Figure 1: FTIR Spectrum of Lycopene

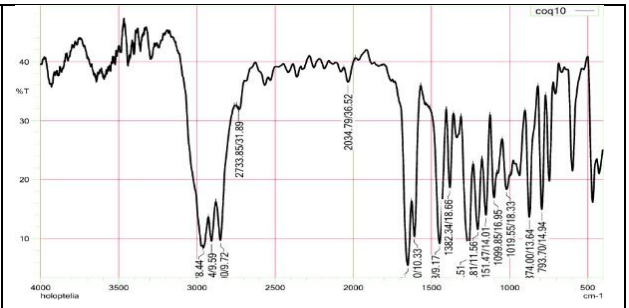

Figure 2: FTIR Spectrum of CoQ10

International Journal of Pharmaceutical Drug Design (IJPDD)
Website: https://ijpdd.org/
ISSN: 2584-2897
Vol. 2, Issue 11, November, 2025
Page No.: 16-24

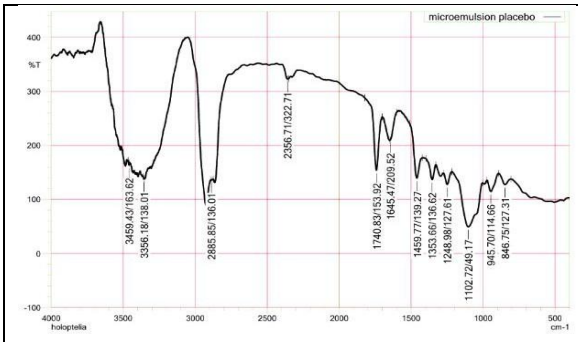

Figure 3: FTIR Spectrum of Microemulsion Placebo

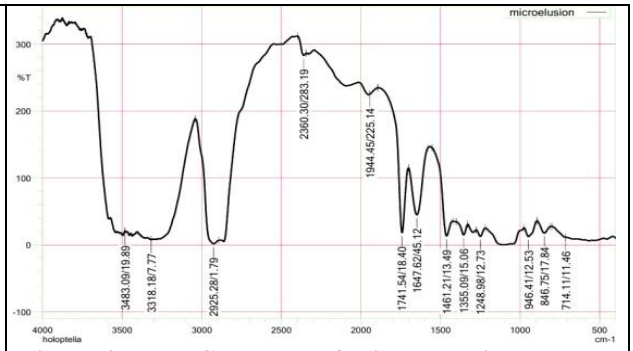

Figure 4: FTIR Spectrum of Microemulsion

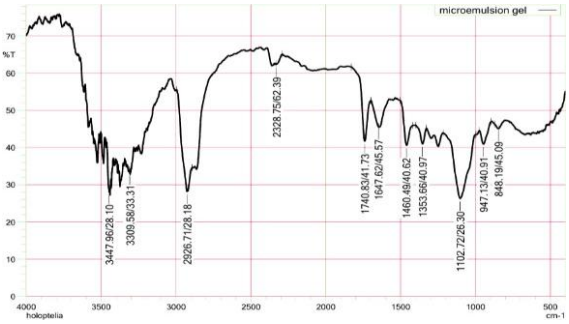

Figure 5: FTIR Spectrum of Microemulsion gel

Particle size:

Increasing the concentration of oil (A) generally resulted in a reduction in particle size, indicating its crucial role in stabilizing the microemulsion system. In contrast, Tween 80 (B) exhibited a parabolic effect on particle size, where moderate levels led to the smallest particles, while both lower and higher concentrations caused an increase in size.

Zeta potential:

For good stability and inhibition of aggregation, a zeta potential value greater than ±30 mV is generally required. In this study, the zeta potential for Lycopene and Coenzyme Q10-loaded microemulsion ranged from –32 to –35 mV, indicating sufficient surface charge to provide electrostatic repulsion and ensure good colloidal stability of the system.

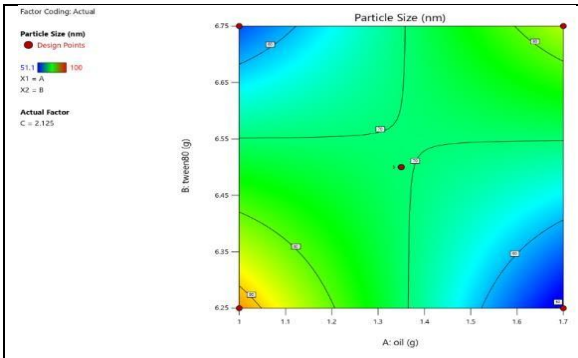

Figure 6: Contour plot depicting the effect of the amount of Concentration of total oil and concentration of total Surfactant on Particle size

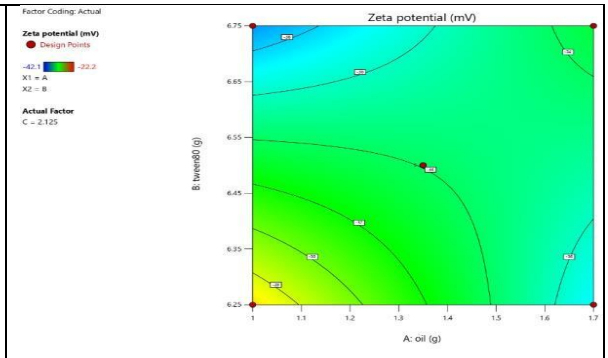

Figure 7: 3D Surface response plot of particle size

International Journal of Pharmaceutical Drug Design (IJPDD)
Website: https://ijpdd.org/
ISSN: 2584-2897
Vol. 2, Issue 11, November, 2025
Page No.: 16-24

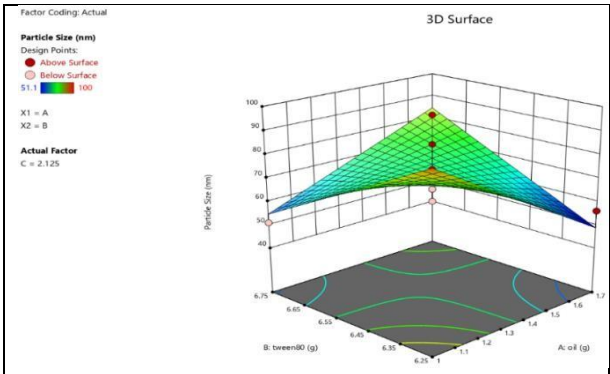

Figure 8: Contour plot depicting the effect of the amount of oil and surfactant on Zeta potential.

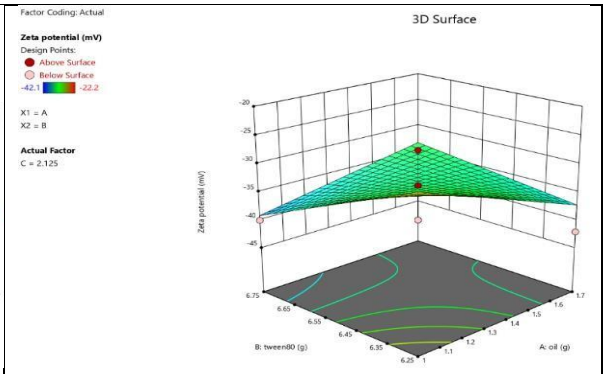

Figure 9: 3D Surface response plot of zeta potential

Polydispersity Index (PDI):

PDI is a unitless parameter that reflects the polydispersity of the formulation. In this study, the Lycopene and Coenzyme Q10-loaded microemulsion exhibited low PDI values, indicating a uniform and homogeneous particle population. The combination of an appropriate particle size with low PDI confirmed acceptable monodispersity, which is a critical factor in ensuring the stability and suitability of the formulation for final application.

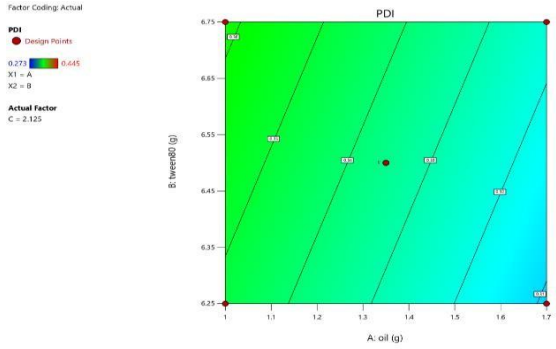

Figure 10: Contour plot depicting the effect of the amount of oil and surfactant on PDI.

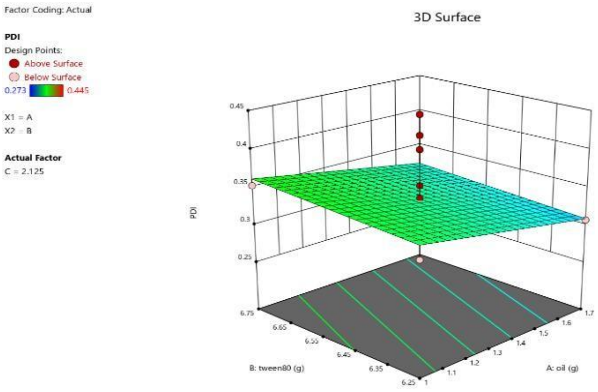

Figure 11: 3D Surface response plot of PDI

Entrapment Efficacy of Lycopene:

The interaction between oil (A) and Tween 80 (B) showed that entrapment efficiency (EE) was low at high surfactant with low oil, but improved when both were present in moderate amounts. Maximum EE was achieved around 1.3–1.5 g oil and 6.4–6.5 g Tween 80, where a balanced ratio provided stable droplets and better lycopene solubilization.

International Journal of Pharmaceutical Drug Design (IJPDD)
Website: https://ijpdd.org/
ISSN: 2584-2897
Vol. 2, Issue 11, November, 2025
Page No.: 16-24

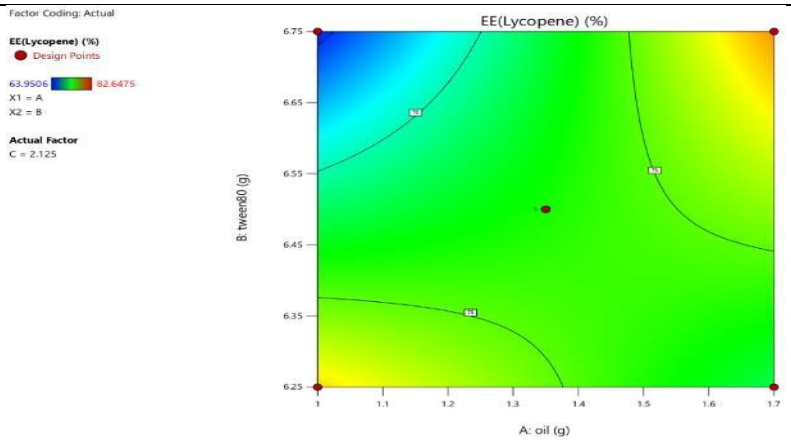

Figure 12: Contour plot depicting the effect of the amount of oil and surfactant on Entrapment Efficiency (Lycopene).

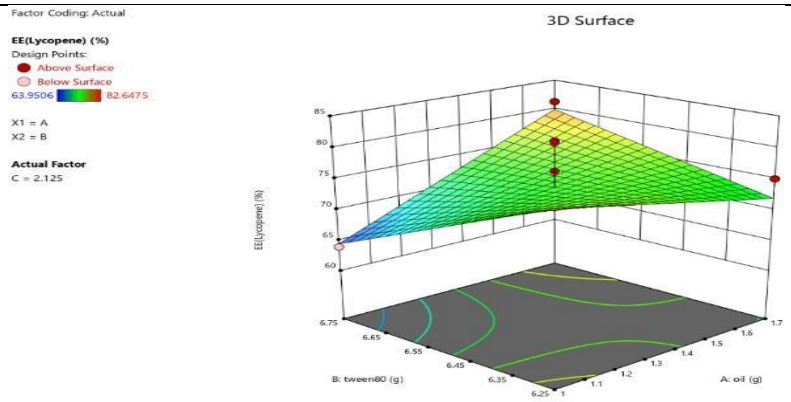

Figure 13: 3D Surface response plot of Entrapment Efficiency (Lycopene).

Entrapment efficacy of CoQ10:

The entrapment efficiency of CoQ10 remained consistently high (94–99%) across all formulations, showing only slight improvement with increasing oil and Tween 80. This indicates efficient incorporation of CoQ10 into the microemulsion with minimal dependence on formulation ratios.

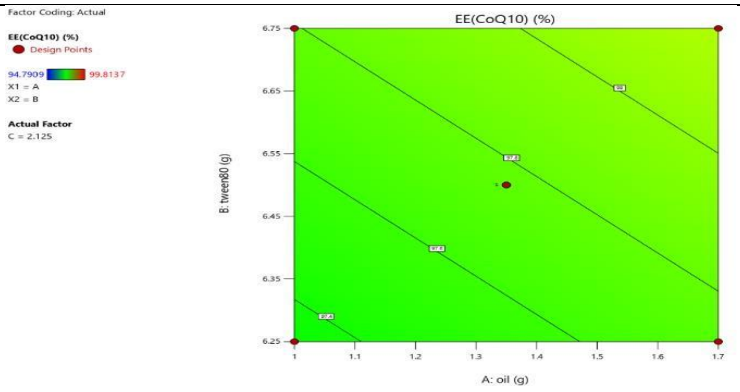

Figure 14: Contour plot depicting the effect of the amount of oil and surfactant on Entrapment Efficiency (CoQ10).

International Journal of Pharmaceutical Drug Design (IJPDD)
Website: https://ijpdd.org/
ISSN: 2584-2897
Vol. 2, Issue 11, November, 2025
Page No.: 16-24

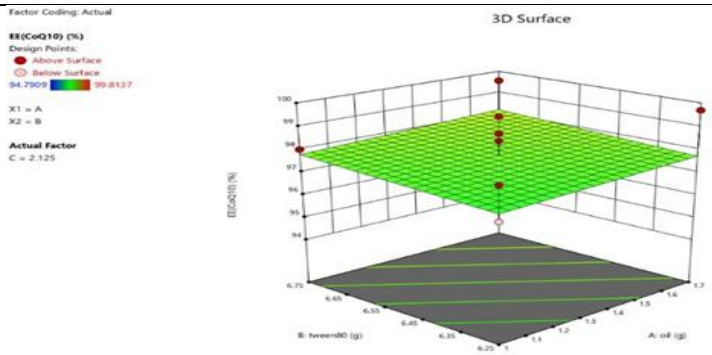

Figure 15: 3D Surface response plot of Entrapment Efficiency (CoQ10).

In vitro drug release :

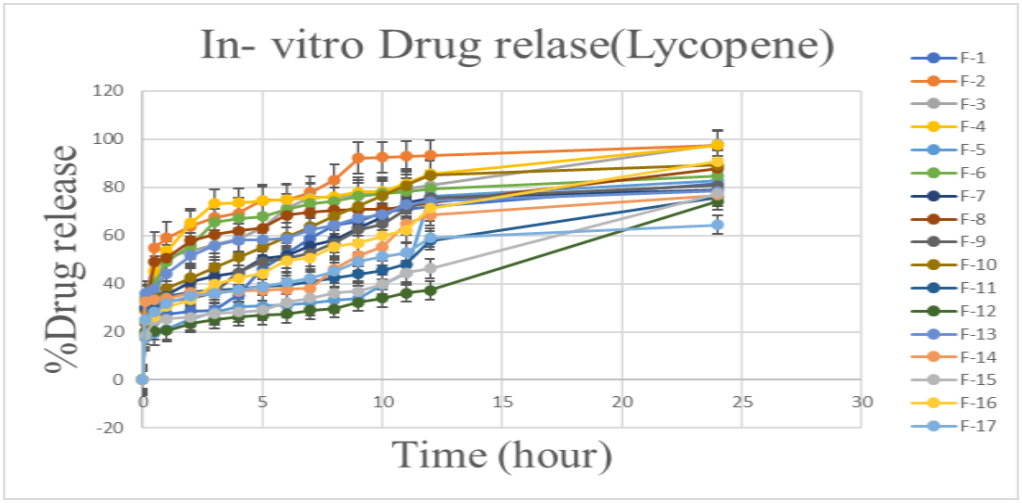

Figure 16: Drug Release of Lycopene of All the Formulations using BBD

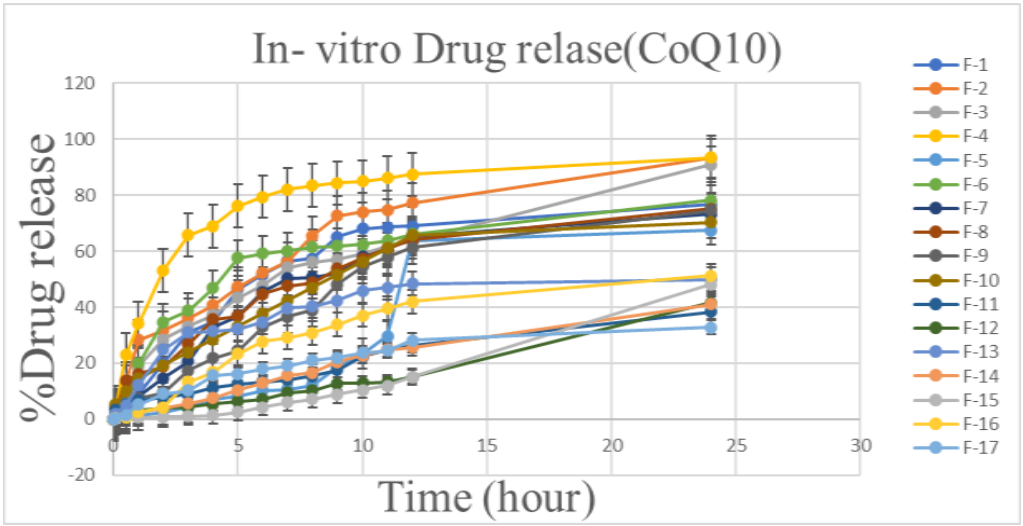

Figure 17: Drug Release of CoQ10 of All the Formulations using BBD

Table 3: Characterization of optimized microemulsion

Particle Size(nm)	52
Zeta potential(mV)	-31.9
PDI	0.366

Entrapment efficacy (Lycopene) (%)	74.543
Entrapment efficacy (CoQ10) (%)	97.5155
Drug release (Lycopene)1Hr (%)	55.9383
Drug release (CoQ10)1Hr (%)	28.8043
Drug release (lycopene)5Hr (%)	77.8487
Drug release (CoQ10)5Hr (%)	62.3068
Drug release (Lycopene)12Hr (%)	90.358
Drug release (CoQ10)12Hr (%)	76.2733
Drug release (Lycopene)24Hr (%)	97.8542
Drug release (CoQ10)24Hr (%)	90.4503

Table 4: Characterization of optimized microemulsion gel

ph	6.5
Viscosity(cp)	128800
Spreadibility	17.875gm.cm/s

Table 5: %Drug release in goat mucosal skin

Time	% Drug release (Coq10)	% Drug release (Lycopene)
0	0.0000	0.0000
0.08	0.3416	22.4691
0.5	10.7143	42.2395
1	23.1366	51.3580
2	29.5963	54.8765
3	31.0559	59.5556
4	37.8882	64.8710
5	43.6733	75.2469
6	50.3106	78.3333
7	57.9193	80.3704
8	65.8385	81.7284
9	68.3230	83.1481
10	72.0497	84.7531
11	73.5404	85.9877
12	74.4720	89.1358
24	80.5901	96.1111

CONCLUSION

The optimized Lycopene and Coenzyme Q10-loaded microemulsion demonstrated nanoscale size, high stability, efficient drug entrapment, and sustained biphasic release. These properties make the system capable of reducing oxidative stress, improving tissue flexibility, and easing oral discomfort. Overall, the developed microemulsion shows strong potential as a safe and effective treatment for OSMF and may also be useful in other oral conditions linked to oxidative stress.

REFERENCES

1. Ku JC, Raiten J, Li Y. Understanding fibrosis: mechanisms, clinical implications, current therapies, and prospects for future interventions. Biomedical Engineering Advances. 2024 Jun 1;7:100118.
2. Chhabra AK, Sune R, Reche A. Oral submucous fibrosis: a review of the current concepts in management. Cureus. 2023 Oct 18;15(10).
3. Swain N. Medical management of oral submucous fibrosis: An update. Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology. 2013 Apr 1;25(2):151-6.
4. Shafe MO, Gumede NM, Nyakudya TT, Chivandi E. Lycopene: a potent antioxidant with multiple health benefits. Journal of

nutrition and metabolism. 2024;2024(1):6252426.
5. Sifuentes-Franco S, Sánchez-Macías DC, Carrillo-Ibarra S, Rivera-Valdés JJ, Zuñiga LY, Sánchez-López VA. Antioxidant and anti-inflammatory effects of coenzyme Q10 supplementation on infectious diseases. InHealthcare 2022 Mar 7 (Vol 10, No. 3, p. 487). MDPI.
6. Umar O, Kumar K, Joshi A, Khairiya D, Teotia D, Ikram I. A comprehensive review on microemulsions: a potential novel drug delivery system. International Journal of Indigenous Herbs and Drugs. 2022 May 29:56-61.
7. Soni PK, Saini TR. Formulation design and optimization of cationic-charged liposomes of brimonidine tartrate for effective ocular drug delivery by design of experiment (DoE) approach. Drug development and industrial pharmacy. 2021 Nov 2;47(11):1847-66.
8. Luthra S, Kalonia DS, Pikal MJ. Effect of hydration on the secondary structure of lyophilized proteins as measured by Fourier transform infrared (FTIR) Spectroscopy. Journal of Pharmaceutical Sciences. 2007 Nov 1;96(11):2910-21.
9. Sopyan I, Gozali D, Paramudya E. Formulation and Stability Testing of Griseovulfin Microemulsion. Indonesian Journal of Pharmaceutics. 2020;2(2):34-42.
10. Khan MF, Singh MK, Sen S. Measuring size, size distribution, and polydispersity of water-in-oil microemulsion droplets using fluorescence correlation spectroscopy: comparison to dynamic light scattering. The Journal of Physical Chemistry B. 2016 Feb 11;120(5):1008-20.
11. Moghimipour E, Salimi A, Eftekhari S. Design and characterization of microemulsion systems for naproxen. Advanced Pharmaceutical Bulletin. 2013 Feb 7;3(1):63.
12. Zeng Z, Zhou G, Wang X, Huang EZ, Zhan X, Liu J, Wang S, Wang A, Li H, Pei X, Xie T. Preparation, characterization, and relative bioavailability of oral elemene o/w microemulsion. International journal of nanomedicine. 2010 Sep 7:567-72.
13. D'Souza S. A review of in vitro drug release test methods for nano-sized dosage forms. Advances in pharmaceutics. 2014;2014(1):304757.

*****