

**DEVELOPMENT AND EVALUATION OF DOXYCYCLINE LOADED
TEA TREE OIL BASED NANOEMULGEL FOR PERIODONTITIS****Hafsa Mohammedi*, Madhu**

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Article Received on 15 Sept. 2026,

Article Revised on 05 Oct. 2026,

Article Published on 16 Oct. 2026,

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College of Pharmacy, Raichur-584103.
Karnataka, India.**How to cite this Article:** Hafsa Mohammedi*,
Madhu (2026). Development And Evaluation Of
Doxycycline Loaded Tea Tree Oil Based
Nanoemulgel For Periodontitis Disease. World
Journal of Pharmaceutical Research, 15(20),
838-854.This work is licensed under Creative Commons
Attribution 4.0 International license.**ABSTRACT**

The subgingival microenvironment in periodontitis presents an aggressive physiological barrier due to the constant upward flow of gingival crevicular fluid (GCF). Establishing a prolonged, controlled retention system inside the periodontal pocket is essential for clinical success. This study aimed to develop a thermoreversible in situ nanoemulgel loaded with Doxycycline (DOX) and Tea Tree Oil (TTO) to achieve targeted, sustained anti-infective activity. O/W nanoemulsions were prepared via high-shear mechanical stirring and ultrasonication using TTO as the lipid core, Tween 20 as a surfactant, and Propylene Glycol (PG) as a co-surfactant. The optimized nanoemulsion (F3) was incorporated into a thermoreversible in situ gelling matrix composed of Poloxamer 407 (15% w/v) and HPMC K100M (5% w/v). Optimized

nanoemulsion droplets exhibited an average vesicle size within the ideal submicron range, satisfactory zeta potential, and narrow polydispersity. In situ gelation transitioned perfectly at simulated periodontal temperature (38.09 °C). Ex vivo permeation studies across goat oral mucosa demonstrated a highly controlled, non-Fickian anomalous diffusion pathway (99.46 ± 0.04% release over 12 hours) compared to immediate exhaustion from plain gels (97.73 ± 0.15% at 6 hours). The study concludes that the developed in-situ nanoemulgel offers a sustained, controlled drug release profile through the synergistic action of the nanoemulsion's oil globules and the dense polymeric matrix of the in-situ gel minimizes total dose requirements, reduces dosing frequency, and enhances patient compliance.

1. INTRODUCTION

Periodontitis is an immuno-inflammatory disease characterized by the destruction of supporting periodontal tissues surrounding teeth in the oral cavity. The subgingival pocket creates a favorable ecological niche for anaerobic microbial pathogens like *Porphyromonas gingivalis*.^[1-3] Traditional oral systemic antibiotic treatments often yield inadequate localized antibiotic flux in the gingival crevicular fluid (GCF) and trigger undesirable systemic tracking side effects such as gastrointestinal distress, photosensitivity, and microbiological resistance.

Local drug delivery placed directly into the periodontal pocket provide distinct advantages by localized high drug concentration while lowering therapeutic indices elsewhere. Nanoemulsions constitute submicron isotropic dispersions of oil and water stabilized by surfactant systems.^[4-6] They represent an excellent lipid vehicle choice to facilitate the payload capacity of lipophilic molecules. However, low viscosity profile compromises local clearance behaviors under mechanical salivary rinsing pressures. To overcome early physiological clearance, formulation hybrids known as *In situ* nanoemulgels^[7,8] have emerged. By incorporating nano-droplets into responsive hydrophilic polymer systems showing phase transition (sol-to-gel conversion) upon physical stimulus triggers like temperature alterations, optimized drug presentation profiles can be accomplished. Doxycycline (DOX) represents a second-generation tetracycline with well-established broad spectrum antimicrobial performance alongside sub-antimicrobial tissue protective characteristics.^[9,10] Tea Tree Oil (TTO), an essential oil from *Melaleuca alternifolia*, offers complementary multi-target anti-inflammatory, antibacterial, and tissue repair enhancements through active terpinen-4-ol components.^[11,12] This work outlines the rational fabrication, design of experiments (DoE) optimization, structural properties, and comparative mucosal permeation kinetics of a hybrid DOX-TTO nanoemulgel matrix targeting periodontitis therapy. The synergistic combination of TTO and DOX within a dual-barrier nanoemulgel matrix offers a compelling option to minimize systemic drug side effects, reduce dosing frequency, and enhance patient compliance in periodontitis clinical care.

2. MATERIALS AND METHODS

2.1 Materials

Doxycycline was used as the active pharmaceutical ingredient. Tea Tree Oil was procured from Mohan Oil Mills (Haryana). Tween 20 and Propylene Glycol (PG) were sourced from

Rolex Laboratory Reagent (Bombay) and Nice Chemicals (Cochin) respectively. Poloxamer 407 and HPMC K100M were utilized as gelling polymers.

2.2 Screening of Surfactants and cosurfactant^[13]

Different types of surfactants and cosurfactant were screened for nanoemulsion formulation, which included Span, Tween 20, Tween 60, Tween 80, PG, PEG and ethanol. In water 2.5 mL of 15% w/w surfactant solution was prepared, and fixed volume of oil having highest solubilisation capacity of DOX was added with vigorous vortexing. The addition of the oil was repeated until the solution became cloudy.

Table 1: Screening of Surfactant and Co-surfactant.

Sl. No.	Surfactants	Solubility (mg/ml)	Co surfactants	Solubility (mg/ml)
1	Tween 20	76.01 ± 3.48	PG	60.26 ± 2.10
2	Tween 60	35.23 ± 3.32	PEG400	38.31 ± 0.81
3	Tween 80	26.93 ± 1.13	Ethanol	38.14± 2.94
4	Span 80	23.96 ± 1.50	Glycerine	14.09± 0.24

2.3 Screening of Oil^[14]

The solubility of DOX in various oils was determined by adding an excess amount of drug in 2 ml of the oils (virgin coconut oil, olive oil, flax seed oil, isopropyl myristate, tea tree oil, castor oil, oleic acid) along with selected surfactant and co-surfactant mixture separately in 5-mL-capacity stopper vials., The equilibrated samples were removed from the shaker and centrifuged at 3,000 rpm for 15 min. The supernatant was taken and filtered. The amounts of drug in the filtrates were determined spectrophotometrically using Ultra Violet–Visible (UV–Vis) spectrophotometer after appropriate dilution with absolute ethanol at 274 nm using the corresponding media as blank.

Table 2: Screening of Oils for Nanoemulsion formulaion.

Sl. No.	Oils	Solubility (mg/ml)
1	Tea tree oil	201.95 ± 3.14
2	Flaxseed oil	144.13 ± 1.16
3	Oleic acid	111.5± 1.74
4	Isopropyl Myristate	99.53 ± 2.84
5	Olive oil	41.38± 0.11
6	Castor oil	6.21± 0.71

2.4 Preparation of DOX nano emulsion:^[15,16] Nano emulsion was prepared by ultrasonication method. Surfactant and co-surfactant was mixed in definite ratios, to this S

mix (surfactant mix) 10ml of water was added. The mixture was vortexed for 5 minutes. To this aqueous mixture, oil and drug was added, with stirring for 5 min to get coarse emulsion. The prepared coarse emulsion was stirred continuously with the help of mechanical stirrer for 3 hours and sonicated for 20 minutes.

Table 3: Formulations of Doxycycline Nanoemulsion.

Formulation code	Ingredients Used					S mix ratio	Total Weight (ml)
	API (mg)	TTO (ml)	TWEEN 20(ml)	Propylene Glycol(ml)	WATER (ml)		
F1	100	0.1	0.96	0.24	8.7	4: 1	10
F2	100	0.1	0.80	0.40	8.7	2: 1	10
F3	100	0.1	0.60	0.60	8.7	1: 1	10
F4	100	0.2	0.40	0.80	8.7	1: 2	10
F5	100	0.2	0.30	0.90	8.7	1: 3	10

2.5 Formulation of In-situ nanoemulgel using poloxamer 407 as gel forming polymer:^[18,19] Nanoemul gel is prepared by temperature dependent method. In 5ml of cold optimized nanoemulsion,(F1) 750mg of poloxamer 407 is added and it is dissolved. Then, 250mg of HPMCK100M is added and mixed continuously to dissolve. Then the solution is kept at 4°C in refrigerator for 12 hrs. After 12 hrs the solution is in ice form it turns to solution form. Then the solution is kept in 37°C water to form gel.

2.6 Evaluation of prepared NE^[20,21]

2.6.1 FTIR studies

The FTIR spectra for MEB, and optimized core and compression coated tablets were recorded using BRUKER-FTIR spectrophotometer in the wave number region from 4000 cm⁻¹ to 500 cm⁻¹. The KBr press was used to prepare potassium bromide pellets loaded with samples under the study. Samples and KBr were mixed in a ratio of 1: 100 and pellets were prepared by finely grinding the mixture in a mortar. Finely grinded mixture was introduced into a stainless steel die and pellets were prepared by pressing the die between polished steel anvils at a pressure of 10t/in².

2.6.2 Determination of emulsion type: The emulsion type either oil in water (o/w) or water in oil (w/o) was determined by Dilution test using by water soluble (methylene red) dye. If the emulsion becomes red, then it is (o/w) type of emulsion and vice-versa. The solution was turned red color which indicated that it is o/w type of emulsion.

2.6.3 Dispersibility test: The dispersibility time were evaluated by adding 1 ml of each NE to 200 ml of distilled water maintained at 34°C using a hot plate with magnetic stirring at 50 rpm. The homogeneity of formulations was visually assessed by recording the time needed for incidence of complete nanoemulsification according to the following grades;

- Clear or slight blue emulsion obtained within 1 min. (classified as grade A)
- Less clear or bluish white emulsion obtained within 1 min. (classified as grade B)
- Milky white emulsion obtained within 2 min. (classified as grade C)
- Dull and greyish white emulsion obtained after 2 min having oily appearance and slower emulsification. (classified as grade D)
- Formulation having large oil globules on the surface and poor emulsification. (classified as grade E)

2.6.4 THERMODYNAMIC STABILITY TESTING

For thermodynamic stability testing, each NE was diluted to 50 times with distilled water and then following tests were performed on prepared nanoemulsion formulations.

Centrifugation test: For centrifugation, all formulations were filled in separate centrifuge tubes and were centrifuged at 3500 rpm for 20 min. Then all formulations were visually observed for phase separation and creaming.

Heating cooling cycle (H/C cycle) test: Formulations were subjected to heating cooling cycle. All formulations were stored at temperature 4^o C and 45^o C for 24hrs. After this, formulations were observed for any instability (phase separation, turbidity etc).

pH: The pH values of the selected NE formulations were determined by dipping the glass electrode of a digital pH meter into a 50 ml beaker containing a sufficient quantity of each prepared formulation at room temperature.

2.6.5 Size and morphology^[22,23]

Spread a thin layer of formed nanoemulsion on a glass slide and observed the structure of vesicle under microscope, collected the images using digital camera. In each case vesicle size analysis was carried out using Malvern Zetasizer Nano ZS (Malvern Instruments, UK). Polydispersity Index (PDI) was also determined as a measure of homogeneity. Zeta potential of the nanoemulsion formulation was determined to estimate stability of the formulations.

2.6.6 Encapsulation efficiency (% EE): The separation of unencapsulated (free) drug from the Doxycycline loaded nanoemulsion globules was achieved via indirect centrifugation. Briefly, a 2 mL aliquot of the prepared DOX dispersion was transferred to centrifuge tubes and subjected to centrifugation at 15,000 rpm for 30 minutes. Following centrifugation, the supernatant containing the untrapped drug was carefully isolated and quantitatively diluted with phosphate buffer solution (pH 6.8). The concentration of free DOX in the supernatant was determined spectrophotometrically by measuring the absorbance at a maximum wavelength of 274 nm utilizing a UV-Visible spectrophotometer. The mass of encapsulated DOX was calculated by subtracting the concentration of unencapsulated drug found in the supernatant from the total initial amount of drug added to the formulation. The percentage encapsulation efficiency (EE%) was computed using Equation:

$$\% \text{ Encapsulation efficiency (EE)} = \frac{\text{Total drug} - \text{free drug}}{\text{Total drug}} \times 100$$

2.6.7 In vitro diffusion study of nanoemulsion^[24,25]

In vitro release studies on DOX nanoemulsion were performed using Franz-diffusion cell. The dialysis cellophane membrane was mounted between the donor and receptor compartment. The receptor medium was phosphate saline buffer pH 6.8. A weighed amount of nanoemulsion preparation was placed on one side of the dialysis membrane. The receptor compartment was surrounded by a water jacket to maintain the temperature at 37 ± 1 °C. A Teflon-coated magnetic bead operated to a magnetic stirrer stirred the receptor fluid. Periodically remove the samples and replaced immediately with an equal volume of receptor media, further drug content was determined spectrophotometrically at 274 nm. Compute the In vitro drug release data by using PCP Disso V3.

2.7 Evaluation of prepared in-situ gel^[26]

2.7.1 Determination of Sol -Gel transition temperature

The gelation temperature was evaluated via the test tube tilting method by placing 2 mL of the formulation into a sealed, 1 cm diameter test tube. The sample was equilibrated in a 4 °C water bath before undergoing stepwise heating. The temperature was increased by 3 °C initially, and then by 1 °C increments as it approached 30 °C, maintaining each temperature stage for 10 minutes. The sol-gel transition point was defined and recorded as the temperature at which the formulation ceased to flow when the tube was tilted by 90°C.

2.7.2 Ex-vivo permeation study: Ex-vivo permeation was carried out using Goat Oral Mucosal tissue.^[27] The ex-vivo permeation was conducted by using Franz diffusion cell, the Goat Oral Mucosal tissue was mounted between the donor and receptor compartment with the mucosal membrane facing upper side on the diffusion cell. The diffusion area of the cell was 1.41 cm² and 25 ml phosphate buffer pH 6.8 taken in receptor compartment, temperature maintained at 37 ± 1°C. DOX loaded nanoemulgel equivalent to 20 mg was spread over the Goat Oral mucosal tissue. The receptor compartment content was stirred with the help of magnetic beads. The samples were collected at different time intervals and were immediately replaced with the fresh media. The samples were analyzed for drug content using UV spectrophotometer at 274 nm. The amount of drug permeated at different time intervals and relevant parameters was calculated.^[28]

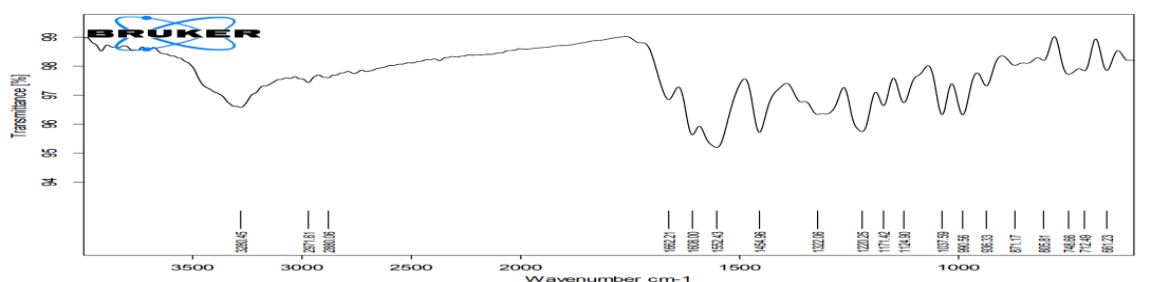
2.8 Stability studies^[29,30]

The short stability study was performed according to ICH guidelines. The optimized DOX loaded nanoemulgel were filled in tightly closed glass vials and subjected to stability testing. The formulations were kept at refrigerated conditions (4±1°C) and at room temperature (25±2°C) and were analyzed for vesicle size, drug content and entrapment efficiency after 3 months.

3. RESULTS AND DISCUSSION

3.1 FTIR studies

The FTIR spectrum recorded for DOXCY and optimized DOXCY gel were depicted in figure 1. The characteristic FTIR peaks of Doxycycline were found at 3008.49 cm⁻¹ corresponding to Ar-CH=CH stretching of CH₃, 1506.14 cm⁻¹ corresponds to N-H bending vibration of amide. Characteristic FTIR peaks of DOXCY were found in optimized DOXCY gel indicates no interaction.



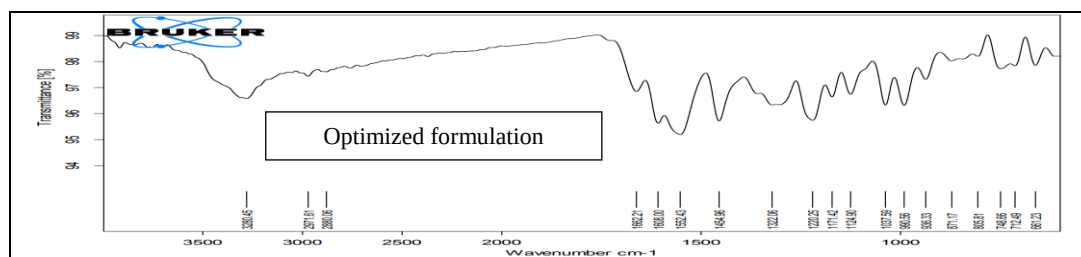


Figure 1: FTIR spectra of DOX and nanoemulsion.

3.2 Appearance

Visual inspection of the formulated nanoemulsion-based gels revealed a highly homogeneous, translucent appearance with a smooth, viscous texture and no evidence of phase separation, precipitation, or flocculation. The pH values of the formulations, determined using a calibrated digital pH meter, ranged from 5.6 to 6.1 (Table 4), which falls within the biocompatible range for topical or mucosal application. The percentage of drug content across the formulations ranged between $67.9 \pm 0.0023\%$ and $94.0 \pm 0.0015\%$. The remarkably low standard deviation values confirm excellent drug distribution uniformity and structural stability within the nanoemulsion network.

Table 4: The physical appearance, pH, % entrapment efficiency percentage (% EE) and, particle size, DOX loaded nanoemulsion.

Batches	Physical appearance	pH(n=3)	Particle size (μm)	% EE $\pm$ SD	PDI
B1	Translucent and homogenous	5.8	3.231 ± 0.0121	93.84 ± 0.0131	0.547
B2	Translucent and homogenous	5.6	3.613 ± 0.0211	83.53 ± 0.1212	0.612
B3	Translucent and homogenous	6.1	4.262 ± 0.0091	76.34 ± 0.0112	0.307
B4	Translucent and homogenous	5.9	5.633 ± 0.0131	72.76 ± 0.0231	0.584
B5	Translucent and homogenous	6.0	6.633 ± 0.0131	68.76 ± 0.0231	0.389

3.3 Particle size and Encapsulation efficiency

Increasing the S mix concentration decreases the average particle size. The average particle diameter of the DOX-loaded nanoemulsion was found to be in the range of 3.2–6.6 μm (Figure 2), characterized by discrete, non-aggregated, and non-agglomerated vesicles. Higher concentrations of S mix provided rapid coverage of newly formed droplets during emulsification, reducing interfacial tension and preventing droplet coalescence. This successfully shrunk the mean particle diameter down to the low nanoscale.^[31,32] For the hydrophilic active compound, rising S mix levels directly expanded the capacity of the droplet interfacial layers. The increased surface area of the smaller droplets provided abundant hydrophilic/polar domains (via surfactant head groups) to securely lock in the water-soluble

drug molecules, resulting in high encapsulation values.^[33] The effect of varying the surfactant-to-co-surfactant S mix ratio (from 4: 1 to 1: 3) on the particle size and encapsulation efficiency (EE) of the DOX-loaded nanoemulsions (hydrophilic API) was systematically studied. It was observed that formulations containing higher proportions of the primary surfactant, Tween 20 (F1 with a 4: 1 ratio), yielded the smallest mean particle size and the highest encapsulation efficiency. Decreasing the Tween 20 concentration while increasing Propylene Glycol reduced the interfacial film's elasticity and mechanical strength. This compromised the system's ability to maintain ultra-small nanodroplets, resulting in a progressive increase in particle size from F1 to F5.^[34] The encapsulation efficiency for the hydrophilic API was optimal at higher surfactant fractions. As the ratio transitioned toward 1: 3 (F5), the reduction in Tween 20 head groups diminished the available interfacial binding sites for the water-soluble drug. Furthermore, excess Propylene Glycol alters the polarity of the external water phase, driving the hydrophilic API out of the nanodroplet boundary and into the continuous phase, lowering total encapsulation.^[35,36]

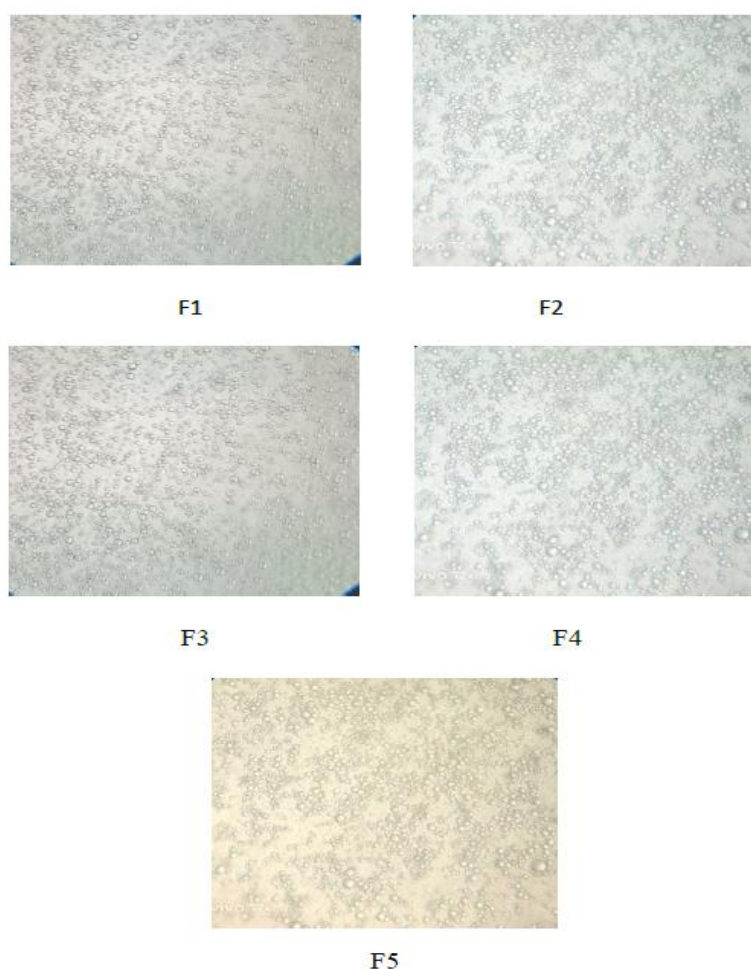


Figure 2: Microphotographs of DOX loaded Nanoemulsion formulations.

3.4 Polydispersity Index and Zeta Potential Analysis

The polydispersity index (PDI) defines the particle size distribution heterogeneity, where values below 0.05 represent highly monodisperse standards and values up to 0.7 indicate a narrow, uniform distribution compliant with ISO standards. The optimized formulation exhibited a PDI of 0.650, confirming a narrow particle size distribution suitable for transdermal drug delivery. Concurrently, the formulation demonstrated a zeta potential of -40.4 mV. Colloidal systems with zeta potentials exceeding ± 30 mV possess strong electrostatic repulsive forces that prevent particle aggregation. The highly negative zeta potential achieved here, driven by the high surface energy of the nanostructures, confirms excellent physical stability for the optimized formulation.

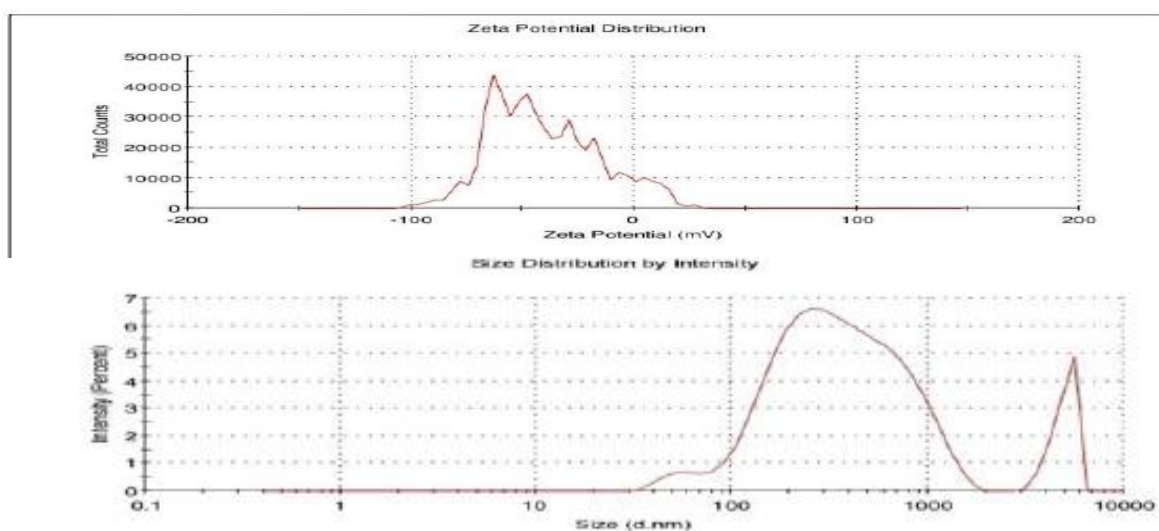


Figure 3: Average diameter and Zeta potential optimized formulation.

3.5 Determination of emulsion type

The emulsion type either oil in water (o/w) or water in oil (w/o) was determined by Dilution test using by water soluble (methylene red) dye. The solution was turned red color which indicated that it is o/w type of emulsion.

3.6 Thermodynamic stability studies

NE formulations possess thermodynamic stability compare to conventional emulsion and this is ascribed not only to the favorable adsorption of surfactant and cosurfactant existing at the o/w interface, but also the avoided coalescence due to reduction of interfacial energy. In order to exclude the possibility of metastable formulations, stress testing was performed for the optimized formulation. The result (Table 5) revealed that all formulation passed the test

without DOX precipitation, phase separation, creaming or even cracking, which justified the stability of the prepared NE.

Table 5: Thermodynamic stability testing.

Formulation	H/C	CENT.	DT Grade	Inference
F1	Stable	No Separation	A (Clear or slight brown emulsion obtained within 1 min)	Passed
F2	Stable	No Separation	A(Clear or slight brown e emulsion obtained within 1 min)	Passed
F3	Stable	No Separation	A(Clear or slight brown emulsion obtained within 1 min)	Passed
F4	Stable	No Separation	A(Clear or slight brown emulsion obtained within 1 min)	Passed
F5	Stable	No Separation	A(Clear or slight brown emulsion obtained within 1 min)	Passed

3.7 *In vitro* release of dox from insitu gel

The drug release from nanoemulsion was indicative of influence of Smix concentration, and was shown in figure 4. The *in vitro* cumulative drug release profiles of DOXCY from formulations **F1 to F5** demonstrated a controlled, composition-dependent release pattern over **12 hours**. All formulations exhibited a characteristic biphasic profile, characterized by an initial minor burst release within the first hour followed by a sustained release phase. The cumulative release rate followed the distinct order of $F5 > F4 > F3 > F2 > F1$. Formulation F5 achieved near-complete drug release (~98%) within 8 hours, while F4 and F3 reached maximum release between **10 and 11 hours**. Conversely, formulations **F2** and **F1** exhibited more prolonged release kinetics, yielding **94%** and **70%** release at **12 hours**, respectively.

Formulation F1 (**Smix**4: 1) exhibited the slowest, most sustained drug release rate. This controlled release profile is attributed to two complementary factors: its ultra-small nanometric droplet size—which results in highly dense packing—and the maximum presence of Tween 20. The abundant surfactant molecules create a rigid, highly condensed interfacial barrier layer that prevents the rapid diffusion of the hydrophilic drug out of the droplet matrix.^[37] As the *Smix* ratio shifted systematically from surfactant-rich to co-surfactant-rich states (**F2 2: 1 to F5 1: 3**), a progressive and significant **acceleration in the cumulative drug release rate** was recorded. Formulation **F5 (1: 3)** demonstrated the **maximum cumulative drug release** over the shortest time interval. This rapid drug release profile occurs because decreasing the primary surfactant concentration reduces the interfacial layer's viscosity and density, creating a more porous and fluid droplet boundary.^[38] The kinetic

studies showed that the release profile followed the zero order, by using Korsmeyer-Peppas equation, the n values obtained <1 for optimized formulation which is characteristic of anomalous kinetics (non-Fickian) and super case-II transport, suggesting that more than one mechanism may be involved in release kinetics, referring to the combination of diffusion and erosion based drug release mechanism.

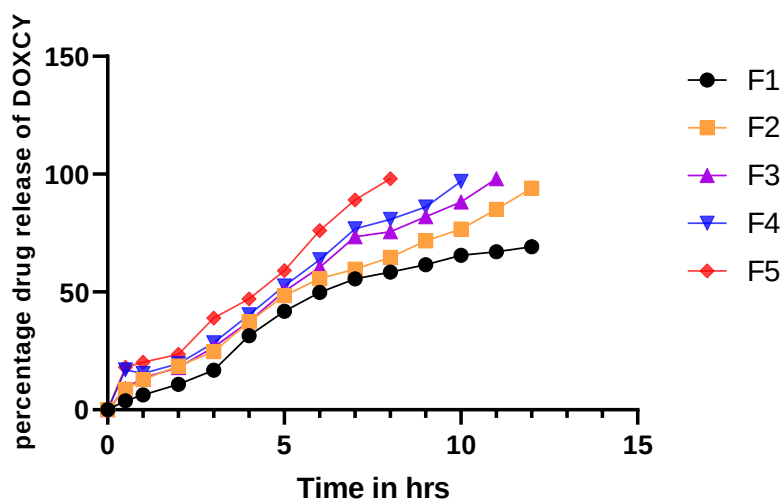


Figure 4: Comparative *In vitro* diffusion profiles of DOX loaded nanoemulsion.

4 Evaluation of *in-situ* nanoemulgel

4.1 Evaluation of Visual appearance, Clarity, pH, and Gelling capacity

All the prepared *in-situ* gelling systems were evaluated for preliminary steps such as visual appearance, clarity, pH, and gelling capacity. These formulations were light yellow in color and clear. The prepared insitugel formulation gelled instantaneously (less than a minute) on contact with Simulated saliva and remained as gel for extended period of time. The pH of the formulations was found to be 6.1 to 6.5 which does not causes irritation when applied on oral mucosa.



Figure 5: Conversion of sol to gel.

4.2 Ex vivo permeation studies

Ex vivo permeation of DOX insitu gel was studied in diffusion test apparatus containing 6.8 pH was used as diffusion medium, where prepared goat buccal mucosa used as a semi-permeable membrane to measure release rates. The cumulative amount of drug permeated across the oral mucosa was found to be $97.73 \pm 0.15\%$ at the end of 6 hour in case of plain insitu gel. While the release of drug from nanoemulgel was found to be $99.46 \pm 0.04\%$ at the end of 12 hour. In case of plain gel the drug is dissolved directly within the hydrophilic matrix and diffuses directly through water-swollen polymer channels, leading to rapid, early exhaustion where as nanoemulgel acts as a two-step hybrid barrier. The drug must first partition across the internal lipid core of the nano-droplets into the aqueous phase before diffusing through the crosslinked polymer grid. This dual-phase architecture acts as a structural brake to prolong permeation.

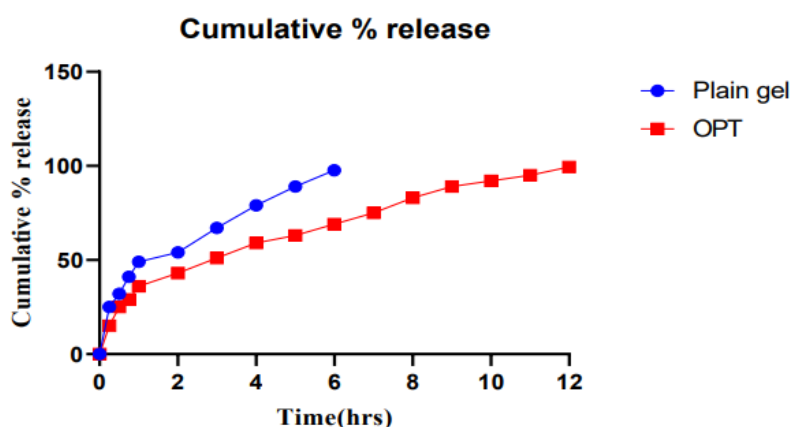


Figure 6: Comparative Graph of Cumulative percent drug permeated data of DOX Plain Gel and DOX nanoemulsion in-situ gel across the Goat Oral Mucosa.

5. Physical stability studies

In the present study, stability studies were performed on DOX loaded insitu nanogel formulation (B1). It was stored at $4^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 3 months and observed for the change in % EE, average PS of the vesicles. It was observed that there was no change in the physical appearance as there was neither change in the formulation consistency nor aggregation of vesicles. Furthermore, there was no significant change in EE%, PS of DOX Nanoemul gel after the storage for 3 months. similarly Hwang J *et al.*^[39] demonstrated that oil-in-water lipid nanoemulsions loaded with doxorubicin retain their encapsulation efficiency and structural integrity without significant vesicle aggregation over a 3-month room temperature storage window. These results demonstrate that the nanoemulgel framework successfully preserves

the nano-architecture of the carrier under storage conditions." The same phenomenon was well documented in recent polymeric nanogel delivery frameworks by Radeva L et al.^[40]

CONCLUSION

DOX-loaded nanoemulsion was prepared by high shear sonication method which is successfully incorporated into temperature sensitive insitu gel. The prepared nanoemulsion exhibited desired entrapment efficiency and Particle size. It was found that F1 which chosen as a best formula according to entrapment efficiency, PS, and *In vitro* release study. *In vitro* diffusion studies conducted on DOX nanoemulsion incorporated insitu gel proved that the prepared formulation considered to be a successful site specific drug delivery system and provide a sustained release of encapsulated drug. Furthermore, there was no significant change in EE% and PS of DOX nanoemulgel after storage for 3 months.

ACKNOWLEDGMENT

We are thankful to the Principal and Management of V. L. College of Pharmacy, Raichur for providing necessary facilities to carry out the work.

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