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Research Article

Design, Optimization, and Characterization of Tranexamic Acid Microemulsion for Topical Delivery

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Abstract

This study focuses on the design, optimization, and evaluation of tranexamic acid (TXA) microemulsions (ME-TXA) for topical delivery to manage melasma. TXA is an antifibrinolytic agent that competitively inhibits plasminogen activation and acts as a non-competitive plasmin inhibitor, reducing melanin synthesis and inflammation in pigment cells. Beyond melasma, TXA has demonstrated potential for treating various skin disorders, including post-inflammatory hyperpigmentation, melanin-related pigmentation disorders [1], and chronic inflammatory conditions such as atopic dermatitis and rosacea [2]. Studies suggest that TXA can be beneficial in treating melasma [3].

The microemulsions were formulated using Labrafac PG/Transcutol P as the oil phase, Tween 80 and Span 80 as surfactants, and propylene glycol (PG) (10:1 ratio) as a co-surfactant These systems offer advantages such as improved drug solubility, enhanced skin penetration, controlled release, and increased formulation stability, making them ideal for topical delivery of TXA [4]. Physicochemical properties, including particle size, viscosity, zeta potential, and permeability, were systematically analyzed. Characterization of the formulations demonstrated particle sizes ranging from 15.08 to 34.75 nm, viscosities between 147.83 and 300.3 cps, and zeta potentials from -1.7 to -13.2 mV, ensuring stability and homogeneity.

In vitro release studies indicated a cumulative drug release of up to 99.3% over 24 hours, following first-order release kinetics. The optimized formulation demonstrated enhanced TXA solubility, stability, and controlled drug release compared to aqueous solutions.

Skin permeation studies revealed significantly enhanced drug permeation, with the optimized formulation achieving a steady-state flux (Jss) of 5.544 mg/cm²·h and a permeability coefficient (P) of 0.1108 cm/h.

In addition, the apparent diffusion coefficient (Dapp) analysis demonstrated a reduced lag time (Tlag) compared to aqueous TXA."

These findings highlight the potential of ME-TXA as a biocompatible and efficient drug delivery system, offering improved solubility, stability, controlled release, and enhanced transdermal permeability for the effective management of melasma.

Keywords: Tranexamic Acid; Microemulsion; Melasma; Optimization; Topical.

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1. Introduction

Tranexamic acid (TXA), chemically known as trans-4-aminomethyl cyclohexane carboxylic acid, is a highly water-soluble synthetic derivative of the amino acid lysine. It functions as a plasmin inhibitor by reversibly blocking plasminogen-binding sites, thereby preventing plasminogen activation and reducing fibrinolysis [5-8]. Initially developed for its antifibrinolytic properties in surgical and trauma-related bleeding, TXA has also demonstrated anti-inflammatory and depigmenting effects, making it a potential therapeutic agent for skin disorders such as melasma.

TXA inhibits melanocyte-keratinocyte interactions by competitively inhibiting tyrosinase activity in melanocytes, thereby reducing melanin synthesis [8-12]. Additionally, due to its structural similarity to tyrosinase, TXA inhibits lipoxygenase activity in human monocytes, thereby reducing inflammation. TXA also modulates UV-induced plasmin activity, mast cell activation, and fibroblast growth factors, ultimately reducing epidermal vascularization and cellular proliferation, contributing to skin-lightening effects [13-16].

Melasma is a chronic hyperpigmentation disorder characterized by symmetric, reticulated dark patches, primarily on the face [17]. Its pathogenesis is complex and influenced by hormonal changes, genetic predisposition, and ultraviolet (UV) light exposure [18]. UV radiation triggers the production of reactive oxygen species (ROS) in keratinocytes and fibroblasts, leading to oxidative stress and increased melanogenesis. Additionally, UV exposure upregulates stem cell factor (SCF) and vascular endothelial growth factor (VEGF), further stimulating melanocyte activity and hyperpigmentation [19].

Current treatments for melasma include topical agents such as hydroquinone, azelaic acid, steroids, kojic acid, and glycolic acid, as well as oral treatments like TXA, melatonin, and cysteamine hydrochloride. However, these therapies have limited efficacy and potential systemic side effects [17, 20].

While some studies have investigated topical TXA formulations, most have focused on conventional creams and gels, with limited research on advanced nanocarrier-based systems, such as liposomes and nanoemulsions, to improve TXA skin permeability [20-23].

Microemulsions (MEs) have emerged as promising drug delivery systems due to their enhanced solubility, bioavailability, and transdermal permeability. These isotropic, thermodynamically stable formulations consist of oil, water, surfactants, and co-surfactants, yielding droplet sizes of 10-100 nm [24].

Compared to nanoemulsions and liposomes, microemulsions require lower surfactant concentrations, are easier to formulate, and can improve drug permeation through the skin barrier [25].

Furthermore, they are non-irritant, non-allergenic, and cost-effective for pharmaceutical applications [26-28].

This study aims to develop and characterize TXA-loaded microemulsions (ME-TXA) as a novel, biocompatible topical delivery system for the management of melasma. By optimizing microemulsion formulations, we seek to enhance TXA's stability, solubility, and skin permeation, offering a more effective and safer alternative to conventional treatments.

2. MATERIALS AND METHODS

2.1. Materials

TXA was purchased from Amin Company (Esfahan, Iran). Tween 80, Span 80, and propylene glycol were provided by Merck (Germany). Labrafac PG and Transcutol-P were obtained as gifts from Gattefossé (Saint-Priest, France) [29].

2.2. Construction of Pseudo-ternary Phase diagrams

Pseudo-ternary phase diagrams were constructed using the titration method to determine the optimal concentration for the boundaries of microemulsions (MEs) without the addition of TXA. The phase diagrams were created at room temperature by blending surfactants, co-surfactants, and oil phases with water. Two separate phase diagrams were prepared using mass ratios of 4:1 and 6:1 for Tween 80:Span 20 and Propylene glycol, respectively.

For each diagram, the surfactant mixture was added to a Labrafac PG/Transcutol-P blend (10:1) at weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. The surfactant and co-surfactant blends were mixed thoroughly using a magnetic stirrer. Afterward, each mixture was diluted with double-distilled water at 25 ± 1°C [29, 30]. The mixture was considered a microemulsion system once it became clear.

2.3. Preparation of TXA Microemulsions

To develop an optimal TXA microemulsion (ME) formulation, Labrafac PG and Transcutol P were selected as the oil phases due to their ability to enhance skin penetration. Tween 80 and Span 80 were chosen as non-ionic, less toxic surfactants, while propylene glycol was used as a cosurfactant due to its low irritancy and high solubility.

A fullfactorial design with three variables at two levels was employed to generate eight different ME formulations (25-29).

The selected values for the surfactant-to-co-surfactant ratio, oil phase percentage, and water phase percentage were as follows:

- Surfactant-to-co-surfactant ratios: 4:1 and 6:1,
- Oil phase percentages: 20% and 40%,
- Water phase percentages: 5% and 7%.

To prepare the TXA microemulsions, the following procedure was followed:

1. Tranexamic acid (TXA) was first dissolved in the oil phase (Labrafac PG/Transcutol P blend) under gentle heating.
2. The surfactant/co-surfactant mixture (Tween 80 and Span 80) was then added to the TXA-oil mixture and stirred thoroughly.
3. The resulting blend was gradually diluted with double-distilled water at 25 ± 1°C while stirring until a clear, homogenous microemulsion was formed.

The formulation was considered a microemulsion once it became transparent, indicating the successful formation of the system**.**

2.4. Solubility of Tranexamic acid

TXA solubility was tested in several oil phases, Isopropyl myristate, Labrafac PG, Labrafac PG+Transcutol P (10:1), Span 80, Tween 80 as surfactants, and Propylene glycol as co-surfactants by dissolving an excess quantity of TXA in 2 ml of each phase and stirring at 37 + 0.5 ºCfor 72 hours. Next, the samples were centrifuged at 3000 rpm for 15 minutes to remove undissolved TXA, and the supernatant was collected. TXA solubility was estimated by measuring the supernatant absorbance at 217 nm using a UV-visible spectrophotometer after dilution with double-distilled water. [31]. The TXA solubility was estimated by analyzing the supernatant using a UV-visible spectrophotometer after dilution with double water at 217 nm [32].

2.5. Zeta potential determination

The Zeta potential of samples was determined using a Zeta sizer (Malvern instrument 1td ZEN3600, UK) within a dielectric constant of 78. Samples were diluted with double-distilled water (10: 1) before *Zeta potential examination*.

2.6. Measurement of Particle size and refractive index

SCATTER SCOPE 1 QUIDIX (South Korea) was utilized to measure the average particle size of samples at 25 ºC and 632 nm. Refractive index (RI) was determined as well by the Quart's refractometer (Belgium) at room temperature.

2.7. Viscosity measurement

Sample viscosity was found at 25ºC using a Brookfield viscometer (DV-II+Pro, Brookfield, USA) with spindle number 34, and a shear rate of 50 at 25 ± 1°C.

2.8. Conductivity measurements

A conductivity meter (Metrohm Model 712) with a cell constant of 1.0 was used to assess the electrical conductivity of the samples.

2.9. Surface tension determination

To examine the surface tension of microemulsion at 25ºC, 5 ml of each sample was placed in a container for 5 minutes and then employed the 4 cm ring of the Torsion balance (WHITE ELEC Model NO. 83944E).

2.10. Determination of pH

We used a pH meter (Mettler Toledo Seven Easy, Switzerland) to estimate ME's pH at room temperature.

2.11. Physical stability study

ME's stability was examined using temperature and centrifuge stability tests. For this aim, the samples were stored at 4°C, 25°C, 37°C, and 75% ± 5% RH for three months. Physicochemical changes, including phase separation, turbidity, and particle size changes, were monitored throughout time and temperature. Also, after centrifuging samples at 15000 rpm for 30 minutes at room temperature, and noting changes in ME homogeneity [33].

2.12. Release study

To assess the drug release rate and permeability parameters, a membrane approach was utilized in this study [34]. This approach used Franz diffusion cells (4.906 cm^2) with a cellulose membrane (cut-off size, 12 kDa), 30 mL phosphate buffer (pH 7.4) in the receptor phase, and 5 g of each ME in the donor phase. The Cellulose membrane was soaked in distilled water for 24 hours before being tested at 25ºC. Then it was clamped between the donor and receptor cells. Externally operated magnetic bars continuously swirled the receptor fluid at 200 rpm and 37ºC. At specific time intervals of 0.5, 1, 2, 3, 4, 5, 6, 7, 8, and 24 hours, a 2 ml sample was taken from receptor compartments and examined spectrophotometrically at 217 nm for drug content. To keep the sink state, an equivalent volume of fresh receptor solution was introduced to the receptor chamber. Finally, samples were examined using a UV-visible spectrophotometer at 217nm, and the results were presented as cumulative drug release percentage vs. time [33]. To explore the kinetic models of drug release, ten models of Zero, First, Higuchi, Pepas, Hixon, Second root of mass, 2/3 root of mass, Weibull, Linear Wagner, and Log Wagner for release findings were fitted, and the model with the highest r2 was chosen as the best model [35].

2.13. Optimization of ME formulation

The suitable amount of each phase in the optimum ME formulation was determined using Minitab 16 software, particle size, and released TXA (R2h) results.

2.14. Permeability experiments

A specially designed vertical diffusion cell, with an effective diffusion area of approximately 4.906 cm^2, was employed to assess in vitro permeation. The receptor compartment was filled with 30 mL of phosphate buffer solution (pH 7.4). Hydrated whole skin samples were placed between the donor and receptor phases of the Franz cell, with the stratum corneum (SC) layer of rat skin oriented toward the donor phase. The donor phase was filled with 5 g of the optimized TXA microemulsion (ME) sample. The diffusion cells were maintained at 37 ± 0.5°C on a heater stirrer, with the receptor phase continuously stirred at 200 rpm using small magnetic stir bars. At predetermined time points (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 24, 32, and 48 hours), 2 mL of the receptor phase was withdrawn and replaced immediately with 2 mL of phosphate buffer solution to maintain sink conditions. The amount of permeated TXA in the optimized ME was quantified using a UV spectrophotometer at 217 nm. Free drug microemulsions and a 3% TXA water solution served as negative and positive controls, respectively [36].

The study involved male adult Wistar rats weighing 200-250 g. The research was conducted with approval from the Animal Ethical Committee at Ahvaz Jundishapur University of Medical Sciences under permit number IR.AJUMS.ABHC.REC.1397.072.

2.15. Data analysis and statistics

The permeability coefficient (P) was determined using the equation Jss=P·C0J_[37] C0 represents the drug concentration in the donor phase. The apparent diffusion coefficient (D_{app}) was calculated using the equation (T_{lag} $=h^2/6D$), where h is skin thickness.

To evaluate the relative enhancement in the permeability parameters of the ME-TXA optimized sample in comparison to the control (3% TXA water solution) permeability parameters, the enhancement ratio (ER) was evaluated as follows:

$$ER = \frac{Permeability\ parameter\ amount\ of\ ME\ formulation}{\text{Permeability parameter amount of control}}$$

All experiments were conducted in triplicate, and the results are expressed as the mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA), with significance assessed at $P < 0.05$.

3. Results and Discussion

3.1. Solubility of TXA in Phases:

The solubility data for TXA are reported in **Table 1** and **Figure 1**. Labrafac PG-Transcutol P exhibited the highest TXA solubility among the tested oils (4.236 ± 0.536 mg/mL). TXA solubility in surfactants was highest in Span 80 (2.528 ± 0.208 mg/mL) and Tween 80 (4.690 ± 0.012 mg/mL). Based on TXA solubility experiments in oil, surfactant, and co-surfactant, as well as pre-formulation research, it was determined that Labrafac PG-Transcutol P, Span 80, Tween 80, and propylene glycol were the most suitable for microemulsion (ME) formation.

Table 1. Solubility of TXA in different oils, surfactants, and co-surfactants (mean±SD, n=3)

Phase type	Excipient type	Solubility(mg/ml)
Oil	Labrafac PG	2.936 ± 0.023
Oil	Iso propyl myristate	3.820 ± 0.044
Oil	Labrafac PG+Transcutol P(10:1)	4.236 ± 0.536
Surfactant	Tween 80	4.690 ± 0.012
Surfactant	Span 80	2.528 ± 0.208
Co-surfactant	Propylene glycol	5.278 ± 0.368

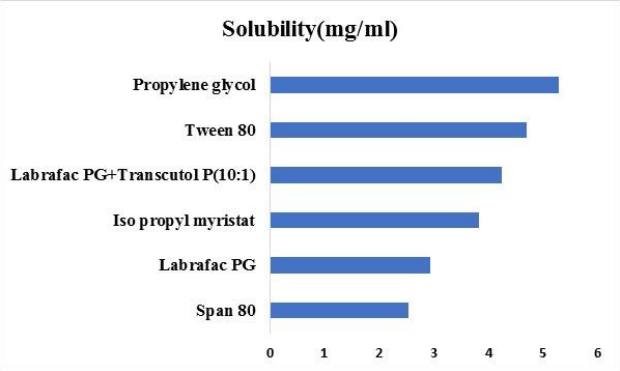

Figure.1 Solubility of TXA in different oils, surfactants, and co-surfactants

The pseudo-ternary phase diagrams of the selected quaternary system (water/Labrafac PG-Transcutol P/Span 80-Tween 80/propylene glycol) are shown in **Figure 2**. The diagrams indicate that the ME formation region expands with an increasing surfactant/co-surfactant weight ratio (Km = 2-6) and a decreasing water percentage. The selected ME formulations and their factorial statuses are listed in **Table 2**.

3.2. Zeta potential:

Zeta potential values of MEs are summarized in **Table 3**, showing an average range from -13.2 to -1.7 mV. ME-TXA-4 had the highest zeta potential, while ME-TXA-7 had the lowest. Regression analysis revealed a significant relationship between zeta potential and both oil percentage (P = 0.003) and surfactant/co-surfactant ratio (P = 0.001). A decrease in the surfactant/co-surfactant ratio and an increase in oil percentage resulted in a reduction in zeta potential, enhancing the stability of the ME formulations [38-40].

This reduction occurs because the droplet surface has a lower charge density, thereby lowering the zeta potential [41].

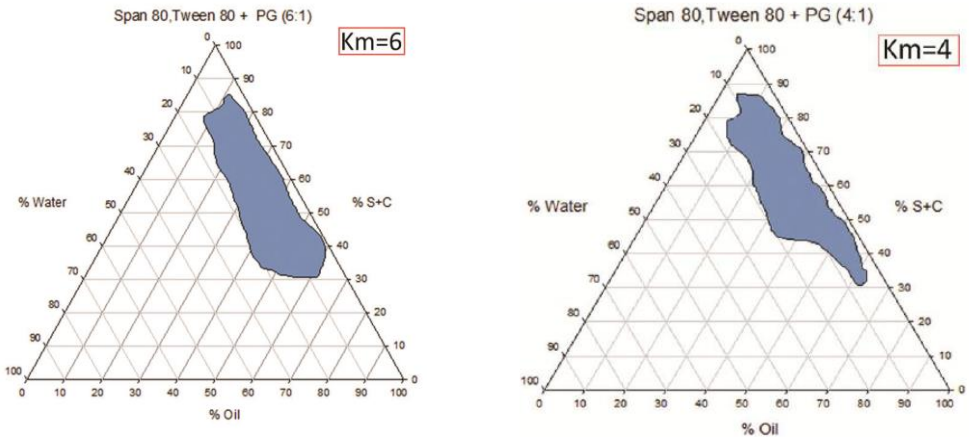

Figure 2. The pseudo-ternary phase diagrams of the oil-surfactant/co-surfactant-water mixture system at 4:1 and 6:1 weight ratios of Span 80/Tween 80/Propylene glycol at room temperature. The dark area shows the ME formation zone.

Table 2. Compositions of Selected ME Formulations

Formulation	Factorial statues	S/C	%S/C	%oil phase	%water phase	%Drug
ME-TXA-1	- + +	4:1	50	40	7	3
ME-TXA-2	- + -	4:1	52	40	5	3
ME-TXA-3	- - +	4:1	70	20	7	3
ME-TXA-4	- - -	4:1	72	20	5	3
ME-TXA-5	+ - -	6:1	72	20	5	3
ME-TXA-6	+ - +	6:1	70	20	7	3
ME-TXA-7	+ + -	6:1	52	40	5	3
ME-TXA-8	+ + +	6:1	50	40	7	3
ME-TXA-Optimize		4.61	59.88	31.39	5.73	3

(+: High level, -: Low level of variables)

Table 3. Zeta potential, Particle size of drug-loaded and drug-free, and viscosity of selected ME-TXA (mean±SD, n=3)

Formulations	Zeta potential(mV)	Particle size(nm) Drug loaded	Particle size(nm) Drug-free	Polydispersity index	Viscosity(cps)
ME-TXA-1	-11±0.09	34.48±0.9	30.1±0.7	0.49	300.3±1.4
ME-TXA-2	-11.1±0.08	20.5±0.5	17.3±0.3	0.31	284.4 ±0.87
ME-TXA-3	-9.98±0.08	34.75±0.8	31.7±0.5	0.49	190.63±1.22
ME-TXA-4	-13.2±0.06	26.85±0.3	22.7±0.4	0.48	174.10±1.2
ME-TXA-5	-10.4±0.09	15.08±0.4	11.2±0.8	0.45	150.4±1.37
ME-TXA-6	-9.89±0.07	17.76±0.6	13.2±0.5	0.47	147.83±061
ME-TXA-7	-1.7±0.05	15.58±0.3	12.4±0.6	0.43	238.63±1.14
ME-TXA-8	-3.03±0.02	26.78±0.3	23.6±0.4	0.47	263.03±1.47

3.3. Particle size

The average particle size of ME formulations ranged from 15.08 to 34.75 nm (**Table 3**). There was no significant difference between free drug MEs and drug-loaded MEs. ME-TXA-5 exhibited the smallest average particle size (15.08 ± 0.2 nm) with a polydispersity index (PI) of 0.45. PI values for all formulations indicated a narrow size distribution. The correlation between particle size and independent factors is expressed by **Equation 1**.

Particle size = 31.1 - 0.256 (oil%) - 3.18 (W%) + 3.93 (s/c) **(Equation 1)** [31]

Where:

- Oil% refers to the percentage of oil in the formulation,
- W% represents the water content percentage,
- s/c stands for the surfactant/co-surfactant ratio.

Statistical analysis revealed a significant association between mean particle size and both the surfactant/co-surfactant ratio (P = 0.015) and water content (P = 0.041). Increased surfactant/co-surfactant ratio and water content decreased the mean particle size, contributing to ME stability [42].

3.4. Viscosity:

Viscosity values ranged from 147.83 ± 0.61 cps to 300.30 ± 1.40 cps, with ME-TXA-6 exhibiting the lowest viscosity (**Table 3**). The relationship between viscosity and independent factors is given by Equation 2.

Viscosity=113+6.75 w% - 18.7 s/c + 5.30 oil% **(Equation 2)**

In this equation, viscosity is expressed as a function of several parameters, including water content percentage (w%), surfactant/co-surfactant ratio (s/c), and the oil percentage (oil%).

It should be noted that the viscosity values reported here represent the average viscosity for each formulation.

A significant correlation ($P \leq 0.05$) was observed between viscosity and both oil percentage and surfactant/co-surfactant ratio. A decrease in oil percentage and an increase in the surfactant/co-surfactant ratio resulted in reduced viscosity.

3.5. Specific structure and conductivity:

The electrical conductivity of ME systems was significantly influenced by their structure [43, 44]. Conductivity values ranged from 0.094 to 2.435 mS/cm, confirming a water-in-oil (w/o) microemulsion structure [42- 44] (**Table 4**).

Water-in-oil microemulsions typically exhibit conductivity values in the range of 1 to 100 µS/, depending on the amount of water in the microemulsion and the presence of dissolved electrolytes or other conductive species in the aqueous phase. Also, the w/o structure of Microemulsions was confirmed with color tests.

Conductivity followed the equation3 :

Electrical conductivity = -0.47 + 0.0353 (oil%) - 0.064 (W%) + 0.180 (s/c) **(Equation 3)**

In this equation, water content percentage (w%), surfactant/co-surfactant ratio (s/c), and oil percentage (oil%) are represented as abbreviations in parentheses.

A significant correlation ($P = 0.06162$) was found between electrical conductivity, surfactant/co-surfactant ratio, and water percentage.

3.6. Surface tension:

The surface tension of water-in-oil (w/o) emulsions typically ranges between 30 and 50 mN/m. However, it can vary depending on factors such as the emulsion composition, the nature of the oil and surfactants used, and the concentration of the surfactant or co-surfactant [45].

The mean surface tension of the formulations ranged from 42 to 46.66 mN/m (**Table 4**), consistent with a water-in-oil microemulsion, as the observed values are close to the oil phase's surface tension.

3.7. pH

The formulations had a suitable average pH value of 6.72±0.05 for topical administration. Incorporating TXA did not significantly influence the pH values of the formulations (**Table 4**). Furthermore, analysis of variance revealed no significant correlation between pH and the independent formulation factors.

Table 4. Conductivity, Surface tension, pH of selected ME-TXA (mean±SD, n=3)

Formulations	Conductivity (ms/cm)	Surface tension (mN/m)	pH
ME-TXA-1	1.44±0.04	44.66±0.06	6.3±0.05
ME-TXA-2	0.68±0.02	42±0.08	6.71±0.08
ME-TXA-3	0.85±0.025	46.66±0.02	6.82±012
ME-TXA-4	0.72±0.021	40.66±.023	6.86±0.09
ME-TXA-5	0.81±0.24	40.66±0.015	6.72±0.09
ME-TXA-6	0.62±0.018	42±0.01	6.76±0.09
ME-TXA-7	2.46±0.73	42.5±0.05	6.82±0.07
ME-TXA-8	1.24±0.37	42.66±0.104	6.81±0.10

3.8. Visual inspection experiment:

The visual assessment investigation lasted three months, with all ME samples collected weekly during the first month and monthly thereafter.

Preparations were visually inspected in clear glass containers for any signs of phase separation, precipitation, or flocculation, facilitating easy assessment of the microemulsion.

Visual examination of microemulsions revealed no signs of phase separation, precipitation, or flocculation. Additionally, no phase separation was observed under stress conditions when centrifuged at 10,000 rpm for 30 minutes [33]. Centrifugation tests confirmed that the microemulsions remained homogeneous. exhibiting good physical stability without phase separation.

3.9. In vitro release:

To investigate the release behavior of tranexamic acid (TXA) through the skin, a cellulose membrane was employed. The cumulative amounts of TXA released were plotted as a function of time (hours) (**Figure 3**). **Table 5** presents the cumulative release percentages after 24 hours along with the kinetics of ME-TXAs.

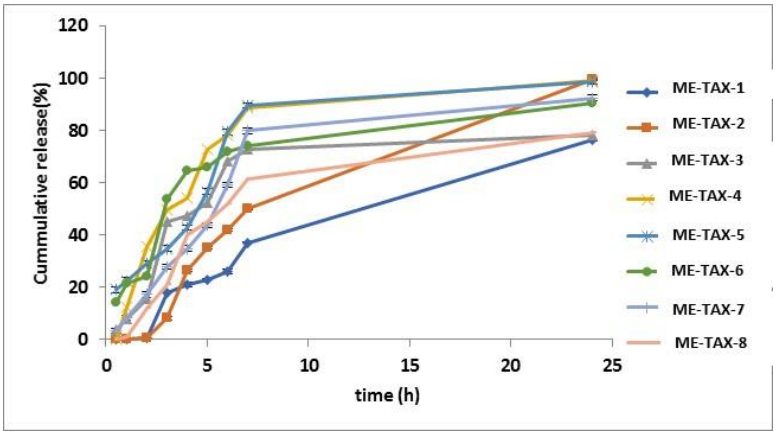

Figure 3. In vitro release profile of ME-TXA formulations

Table 5. Cumulative Release Percentages and Kinetics of TXA Release from Various Formulations of ME-TXA (Mean ± SD, n=3)

Formulation	ME-TXA-1	ME-TXA-2	ME-TXA-3	ME-TXA-4	ME-TXA-5	ME-TXA-6	ME-TXA-7	ME-TXA-8
%TXA release	76.231±1.5	99.453±0.3	78.054±1.1	99..31±0.05	98.723±0.7	90.334±1.1	92.523±1.2	79.271±1.6
Kinetic model	First	Hixon	Log Wagner	First	First	Log Wagner	Weibull	Log Wagner
R^2	0.987	0.9952	0.9954	0.9718	09279	0.6445	0.9629	0.8645

The amount of TXA released varied depending on the ME formulations. ME-TXA-1 exhibited the slowest release, while ME-TXA-4 showed the highest release percentage.

Regression analysis revealed a significant association between drug release percentage and both oil percentage (**P** = 0.041) and water percentage (**P** = 0.01).

Decreasing the oil and water percentages positively influenced the cumulative release percentage.

The influence of independent variables on drug release percentage is described by the linear equation 4.

Drug release percent =141-0.237oil%-8.27W%+0.97 s/c (Equation 4)

Here, water content percentage (W%), surfactant/co-surfactant ratio (s/c), and oil percentage (oil%) are represented as abbreviations in parentheses.

In vitro release studies conducted with an artificial hydrophobic membrane provide valuable insights into a drug's diffusion properties, which depend on the physicochemical characteristics of the formulation components, the vehicle's internal structure, and interactions between the drug and the vehicle [46].

The drug release profile of ME-TXAs was determined by fitting the experimental data to several kinetic models. Linear regression analyses were conducted for zero-order (Mt/M0 = kt), first-order (ln (M0-Mt) = kt), Higuchi (Mt/M0 = (kt) $^{1/2}$), Log Wagner, Linear Wagner, Weibull, Second root of mass, third root of mass, and Pepas kinetics.

The correlation between release kinetics and independent variables showed that as the water percentage increases, the release kinetics shift toward the Log Wagner model. In contrast, decreasing the water percentage shifts the kinetics toward first-order kinetics. The kinetic model remained unaffected by changes in oil content. Additionally, when the surfactant/co-surfactant ratio decreases, the kinetics tend to follow a first-order model, while increasing this ratio shifts the kinetics toward the Log Wagner model.

3.10. Selection of optimized microemulsion:

The optimal formulation was determined using the optimization method in Minitab 16 statistical software, based on droplet size data and the percentage of drug release after 2 hours, which were considered the most valid and effective indicators. Droplet size and drug release were selected as key parameters because they critically impact the formulation's performance and stability.

Specifically, droplet size influences bioavailability and drug absorption rate [47], while drug release over a specified period is essential for evaluating therapeutic efficacy [37].

The optimization process involved calculating the range between the minimum and maximum data points for each variable and targeting these extremes to determine the optimal percentage for each phase. This approach ensured that both particle size and drug release at 2 hours matched the desired target values.

Using Minitab 16®, the software assigns a value of 0 to the least desirable response and 1 to the most desirable. It then calculates the optimal amount for each variable based on the average numerical value of all responses [42]. The optimized component amounts for the ME formulation are reported in **Table 6**.

The target droplet size was set at 25 nm, which is considered optimal for microemulsion formulations, as it typically offers a good balance between stability, drug solubility, and bioavailability [48].

The target for 2-hour drug release was set at 18%, based on the assumption that early-phase drug release is critical for achieving the desired therapeutic effect. This 2-hour release target reflects the need for flexibility in achieving varied release profiles. A value close to 0.1% indicates a slow or negligible initial release, while 35% suggests a rapid initial release. The optimization strategy aims to balance the initial release to ensure it is neither too slow—delaying therapeutic onset—nor too fast—risking potential side effects or toxicity [49].

Table 6. Optimization results and target value

Optimizable Parameter	Minimum	Maximum	Target
Droplet Size (nm)	15	35	25
2-hour release (%)	0.1	35	18

Based on the optimization process, an optimal 3% TXA microemulsion formulation was prepared. Its key characteristics, including droplet size and the percentage of drug released after 2 hours (R_2h), were experimentally measured. The optimized amounts of surfactant/co-surfactant (s/c), oil, and water in the TXA-ME formulation are presented in **Table 2**.

The relationship between droplet size and the independent formulation variables is described by Equation 5.

Droplet Size (nm) = 31.1 - 0.256 (%oil) - 3.18 (%W) + 3.93 (s/c) **(Equation-5)**

Here, "(% oil)" denotes the percentage of oil, "(% W)" refers to the water content, and "(s/c)" represents the ratio of surfactant to co-surfactant.

According to Equation 5, there is a significant relationship between particle size, water percentage, and the s/c ratio. Specifically, a decrease in water content and an increase in the s/c ratio result in a larger droplet size.

Similarly, the equation describing the relationship between the percentage of drug released in 2 hours (R_2h) and the independent variables is given by Equation 6.

2-hour release (R2h) (%) = 47.9 - 0.939 (% oil) - 3.71 (% W) + 3.88 (s/c) **(Equation-6)**

Here, "(% oil)" represents the oil percentage, "(% W)" denotes the water content, and "(s/c)" indicates the surfactant-to-co-surfactant ratio.

According to Equation 6, the 2-hour drug release percentage (R_2h) is significantly influenced by the oil and water content and the s/c ratio. A decrease in both oil and water percentages, along with an increase in the s/c ratio, results in greater drug release at 2 hours.

By substituting the optimized independent variable values into Equations 5 and 6, the predicted values for droplet size and R_2h were calculated and compared with the experimentally measured values. These comparisons, along with regression P-values for each response, are presented in **Table 7**.

Table.7 Properties of ME-TXA- Optimize formulation (mean ± SD, n=3)

Feature	Particle size(nm)	R2h	P value
Actual amount	28.8±0/02	14/85±0/01	0.071
Calculated amount	29.6±0/05	15.1±0/05	0.061

Data are presented as mean ± standard deviation (n=3). *R2h*: Percentage of drug released after 2 hours.

2-hour release (R2h): The actual value of the 2-hour release (R_2h) value was 14.85 ± 0.01, while the calculated value was 15.1 ± 0.05. The P-value of 0.071

indicates that the difference between these values is not statistically significant. Therefore, the actual and computed release percentages are very close, and the slight difference is likely due to random variation rather than an actual discrepancy in the formulation.

Particle Size (nm): The actual particle size measurement was 28.8 ± 0.02 nm compared to a calculated size of 29.6 ± 0.05 nm. The P-value of 0.061 similarly suggests that there is no statistically significant difference between the actual and predicted values. While the particle sizes differ slightly, this difference is insufficient to attribute to formulation or manufacturing variability.

Based on these findings, it can be concluded that the equations relating droplet size and 2-hour drug release to the independent variables are valid. Furthermore, the optimization factor (D = 0.818) for both droplet size and 2-hour release supports the suitability of the optimal formulation for further testing. Additionally, no significant relationship was observed between particle size and 2-hour release in the optimized formulation, as assessed by comparing actual and predicted values (P > 0.05).

The permeation rate of TXA from the optimized microemulsion through whole rat skin was then investigated. The permeation parameters assessed include:

- **Jss** (Steady-state flux, mg/cm²/h): rate of drug permeation at steady state.
- **P** (Permeability coefficient, cm/h): ease of drug passage across the membrane.
- Tlag (Lag time, h): time delay before the drug begins permeating.
- Dapp (Apparent diffusion coefficient, cm²/h): rate of drug diffusion through the membrane.
- ERflux (Effective release flux, mg/cm²/h): rate of drug release from the formulation.
- ERD (Effective release dose, mg: total drug released over time.
- ERP (Effective release percentage, %): percentage of total drug released.

These results are summarized in **Table 8**. To calculate the permeability parameters, a graph of cumulative drug permeated per unit surface area versus time was plotted (**Figure 4**).

As shown in **Table 8**, the permeability parameters for the optimized TXA microemulsion—such as steady-state flux (Jss), permeability coefficient (P), lag time (Tlag), and apparent diffusion coefficient (Dapp)—indicate significantly enhanced permeation across the membrane compared to a TXA aqueous solution. The Jss of the formulated system was markedly higher than that of the water solution, suggesting that the microemulsion facilitates faster drug release and improved absorption.

This enhancement is likely due to excipients, such as surfactants, which increase drug solubility and diffusion, thereby enabling more efficient transport across biological membranes.

Previous studies have demonstrated that propylene glycol accelerates drug distribution within the stratum corneum by slightly disrupting cellular lipid structures, thereby exerting an absorption-enhancing effect similar to that of ethanol.

Surfactants further enhance penetration by dissolving fats in the stratum corneum [50].

Moreover, the combined use of 10% propylene glycol with oleic acid has been reported to amplify this permeation-enhancing effect [51]. Propylene glycol acts by solvating keratin in the stratum corneum and occupying hydrogen-bonding sites, thereby facilitating improved drug penetration [52].

Table 8 Permeability parameters of ME-TXA- Optimize formulation and water solution of TXA

Formulations	Jss(mg/cm².h)	Dapp(cm²/h)	P(cm/h)	Tlag(h)	ER flux	ERD	ERp
water solution of TXA	3.172±0.001	0.0194±0.01	0.063±0.00	2.7824±0.07	-	-	-
ME-TXA- Optimize	5.544±0.90	10.0069±0.03	0.11088±0.018	0.0054±0.61	1.748±0.28	514.2±0.35	1.748±0.28

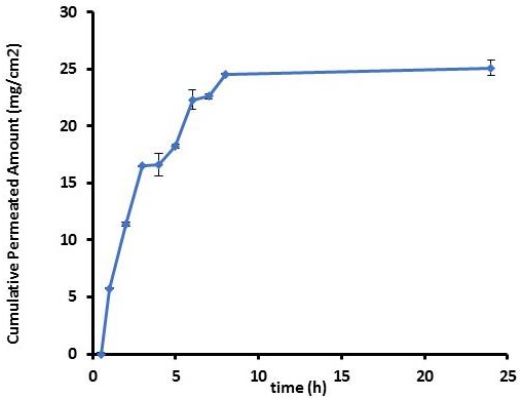

Figure 4. A cumulative amount of TXA permeated through the unit surface area of the whole rat abdominal skin by optimizing the microemulsion

Non-ionic surfactants also modulate drug distribution profiles in the skin [53]. Among them, Span 80 is recognized for its potent skin permeation-enhancing properties [54].

In contrast, TXA in aqueous solution exhibits relatively low permeability due to its hydrophilic nature, limiting its ability to penetrate the skin or other biological barriers. Consequently, drug–membrane interactions are restricted, resulting in a longer lag time (Tlag) and slower release rate, potentially delaying therapeutic effects.

Additionally, the apparent diffusion coefficient (Dapp) for TXA in the microemulsion is higher than that in water. The formulation's smaller drug-loaded droplets penetrate biological membranes more efficiently, improving drug diffusion. This enhanced diffusion likely explains the faster onset of action observed with the formulated product, which is critical for achieving timely therapeutic effects.

Overall, these results highlight the advantages of a formulation-based approach for enhancing the permeability and bioavailability of TXA compared to a simple aqueous solution. The optimized formulation provides a more efficient drug delivery system, ensuring superior drug release and absorption compared to water-based systems. Overall, these results underscore the advantages of a formulation-based approach for enhancing the permeability and bioavailability of TXA relative to simple aqueous solutions. The optimized microemulsion provides a more efficient drug-delivery system, ensuring superior drug release and absorption compared to water-based formulations.

3.11. Discussion

The primary objective of this study was to develop and characterize tranexamic acid (TXA)-loaded microemulsions (ME-TXA) as a novel, biocompatible topical delivery system for the management of melasma. Through formulation optimization, the goal was to enhance TXA's stability, solubility, and skin permeation, thereby providing a more effective and safer alternative to conventional treatments.

The optimized microemulsion significantly improved TXA solubility, particularly by incorporating excipients such as Labrafac PG-Transcutol P and surfactants, including Span 80 and Tween 80. This enhanced solubility enables higher drug loading and improved bioavailability. Zeta potential analysis confirmed better colloidal stability in ME formulations, with optimal surfactant/co-surfactant ratios preventing droplet aggregation and prolonging formulation shelf life.

ME formulations exhibited small particle sizes (15.08-34.75 nm), thereby enhancing drug release, bioavailability, and physical stability. These smaller droplets facilitate better skin penetration and more controlled drug delivery. Additionally, the optimized formulations showed lower viscosity, improving spreadability and ease of application, which are critical factors for patient compliance in topical therapies.

Electrical conductivity studies confirmed that the microemulsion system predominantly had a water-in-oil (w/o) structure, promoting efficient encapsulation and stability of TXA in the oil phase. This arrangement supports controlled release and effective skin

permeation. In vitro release studies demonstrated that the drug release kinetics varied with formulation composition, with the optimized ME achieving a favorable balance between rapid release and sustained delivery—essential for maintaining therapeutic efficacy while minimizing side effects.

Permeation studies further highlighted the superiority of the optimized ME formulation, with significant enhancements in parameters such as steady-state flux (Jss), permeability coefficient (P), and apparent diffusion coefficient (Dapp). The reduced lag time (Tlag) emphasized the formulation's efficiency in accelerating TXA delivery through the skin barrier.

The results also highlight the potential for microemulsions to improve patient outcomes in the management of melasma. TXA-loaded microemulsions offer a non-invasive, well-tolerated alternative with enhanced bioavailability and controlled release properties, potentially paving the way for broader clinical adoption of TXA in dermatology.

This study demonstrates that TXA-loaded microemulsions offer significant advantages in terms of solubility, stability, drug release, and skin permeation, making them a promising alternative for melasma treatment. By addressing current limitations of conventional therapies, ME-TXA formulations have the potential to enhance treatment efficacy and patient adherence, warranting further clinical exploration and development.

4. Conclusion

The primary objective of this study was to develop and optimize tranexamic acid (TXA)-loaded microemulsions (ME-TXA) as an effective, biocompatible topical delivery system for the management of melasma. Through systematic formulation and characterization, the optimized microemulsions demonstrated enhanced TXA solubility, improved stability, and favorable physicochemical properties, including small droplet size and suitable viscosity.

Key findings include significantly improved drug release profiles and enhanced skin permeation parameters, including increased steady-state flux and permeability coefficient, compared with aqueous TXA solutions. The microemulsion system's water-in-oil structure, combined with excipients such as Labrafac PG, Transcutol P, and Span 80, contributed to these improvements by facilitating enhanced drug encapsulation and improved skin penetration.

These results underscore the potential of microemulsion-based formulations to overcome limitations of conventional topical therapies, offering controlled release, higher bioavailability, and improved patient compliance. The optimized ME-TXA formulation represents a promising alternative for melasma treatment with the potential to reduce side effects and improve therapeutic outcomes.

Future research should focus on in vivo and clinical evaluations of safety and efficacy in melasma patients, long-term stability studies under varying environmental conditions, and comparative trials against existing standard treatments. Additionally, mechanistic studies using advanced imaging techniques could elucidate the detailed pathways of TXA microemulsion penetration into the skin. Exploration of personalized formulations tailored to individual skin types may further optimize clinical effectiveness.

Overall, this work lays a solid foundation for the clinical translation of TXA-loaded microemulsions, advancing topical drug delivery strategies in dermatology.

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Conflict of interest

There is no conflict of interest in this study.

Data availability

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Authors Contributions

A.S., and B.SM. conceived and designed the evaluation and drafted the manuscript. A.S., and B.SM. participated in designing the evaluation, performed parts of the statistical analysis. K.N collected the data, interpreted them, and revised the manuscript. Final approval of the version to be published: A.S., B.SM., and K.N.

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Using artificial intelligence chatbots

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