INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

OPEN ACCESS

Research Article

Formulation And Evaluation of Tretinoin Nanoemulgel to Enhance Its Activity

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ARTICLE INFO

Published: 13 Mar. 2025
Keywords:
Nanoemulgel, Nanoemulsion, Bioavailability, Solubility.
DOI:
10.5281/zenodo.15016645

ABSTRACT

Objective: The objective of this study was to develop and optimize a nanoemulgel formulation of tretinoin (TRE) to enhance its topical delivery and therapeutic efficacy for dermatological applications, particularly in treating acne vulgaris. Methods: Tretinoin nanoemulsions were prepared using various oils, surfactants, and co-surfactants. The formulations were optimized through solubility studies, phase diagrams, and physical stability tests. The optimized nanoemulsion was incorporated into a gel matrix to form a nanoemulgel. Characterization of the nanoemulsion included particle size, zeta potential, Transmission Electron Microscopy (TEM), and drug content analysis. The gel was further evaluated for pH, viscosity, spreadability, drug content, and in vitro drug release. The release kinetics were analyzed using different mathematical models. Results: The optimized nanoemulsion, consisting of castor oil, Tween 20, and PEG 400, showed a mean particle size of 53.15 nm and a zeta potential of -22.5 mV, indicating good stability. TEM analysis confirmed spherical to oval-shaped droplets. The nanoemulgel exhibited smooth consistency and good homogeneity without lumps. In vitro drug release studies demonstrated a sustained release profile, fitting best to the Korsmeyer-Peppas model, indicating a combination of diffusion and erosion mechanisms. Conclusions: The nanoemulgel formulation of TRE significantly enhanced the stability and controlled release of the drug compared to conventional formulations. This novel formulation shows potential for improved therapeutic outcomes in the topical treatment of acne vulgaris, providing a promising alternative to existing treatments.

INTRODUCTION

Tretinoin (TRE), also referred to as all-trans-retinoic acid (ATRA), is a naturally occurring compound derived from vitamin A. It is produced as a result of the oxidation process in the metabolic pathway of vitamin A. Within the human body,

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

tretinoin is typically present in very low concentrations, ranging from approximately 4 to 14 nmol/L. This compound possesses several beneficial properties, including anti-inflammatory, antineoplastic, antioxidant, and free radical-scavenging activities. In the field of dermatology, tretinoin has been utilized for numerous years to address various skin conditions, ranging from acne to wrinkles. It functions by activating nuclear receptors, thereby regulating the growth and differentiation of epithelial cells. Tretinoin can be administered orally for the treatment of acute promyelocytic leukemia, and it can also be applied topically to address skin conditions such as acne. [1]. TRE is primarily administered topically due to the severe side effects associated with systemic treatment. The FDA has only approved oral administration of TRE for acute promyelocytic leukemia and moderate to severe cystic acne [2]. Topical application of TRE can cause skin irritation in the applied area. The stability of TRE is limited when exposed to oxygen, light, and acidic pH, resulting in a decrease in effectiveness over time. Its poor solubility in water makes it challenging to incorporate into hydrophilic vehicles. Additionally, existing topical formulations like creams, gels, lotions, and emulsions have limited effectiveness due to skin barrier properties and the relatively low stability of TRE in these products. The degradation of retinoic acid through photo-degradation involves various processes, with isomerization being the most significant. Isomerization transforms TRE into 13-cis retinoic acid (isotretinoin) upon radiation exposure, which further converts into 9-cis retinoic acid. [3]. The primary objective of this study is to create a nanoemulgel containing TRE that has an appropriate and consistent droplet size. Previous research has already established the safety of TRE-NE on healthy human volunteers. In addition, we plan to conduct a preliminary clinical trial to evaluate the therapeutic benefits of the formulation on acne vulgaris lesions, comparing it to the conventional 0.05% TRE nanoemulsion.

Nano-sized drug delivery systems are often favored for the topical administration of medications because they have the ability to offer a controlled release rate and small droplet size, facilitating the efficient deposition of the drug into hair follicles, which is where the drug is most needed. [4]. Drug delivery systems at the nano sized have the potential to improve the stability of pharmaceutical ingredients and enhance their absorption through the skin, ultimately boosting their biological efficacy. These carriers are designed to be safe, capable of holding adequate medication, and facilitating precise and controlled drug release. [5, 6]

MATERIAL AND METHODS

Material

In the experimental study, Tretinoin, obtained from Enaltec Labs in Maharashtra, was used as the Active Pharmaceutical Ingredient (API). A variety of chemicals were sourced from Fischer Scientific and SD Fine-Chem Ltd, both based in Mumbai. Fischer Scientific supplied potassium dihydrogen orthophosphate, sodium dihydrogen orthophosphate, castor oil, oleic acid, Captex 355, and Transcutol HP. SD Fine-Chem Ltd provided methanol, ethanol, DCM, chloroform, olive oil, Labrafil M2125, and n-octanol. Surfactants including Tween 80, Span 80, and Tween 20 were acquired from Molychem, Mumbai, while Kolliphor ELP was sourced from Fischer Scientific. Additionally, propylene glycol, PEG 200, and PEG 400 were supplied by Molychem. These chemicals played a crucial role in the experimental work.

Melting point

The melting point of Tretinoin was determined using the USP technique. Small quantities of Tretinoin were added to a sealed capillary tube, which was then held by the melting point equipment. The temperature of the apparatus was

gradually increased, and the point at which Tretinoin started to melt, as well as the temperature at which it completely melted, were carefully noted. [7].

UV spectrum of tretinoin in methanol

The utilization of a double beam UV-visible spectrophotometer allowed for the determination of the maximum dosage of medication. The scanning process was conducted using a range of 200-600 nm, with a concentration of 7µg/ml in methanol.

Linearity curve of tretinoin using UV-visible spectrophotometer in methanol

A standard stock solution of Tretinoin (100 µg/ml) was prepared using methanol. Initially, 10mg of the drug was dissolved in 100ml of methanol to create a 100ppm stock solution. Subsequently, methanol was utilized to dilute this solution, resulting in dilutions ranging from 1 to 7µg/ml in a UV-visible spectrophotometer. The absorbance of these diluted solutions was then measured at 351nm, with methanol acting as the blank. A standard curve was plotted against concentration, and from this curve, the intercept, slope, straight line equation, and correlation coefficient were determined.

Solubility studies of tretinoin in oils, surfactant and Co-surfactant

The solubility of Tretinoin in different oils, surfactants, and Co-surfactants was assessed by introducing an excess amount of the drug into 2 mL of the specified oils, surfactants, and Co-surfactants (including Olive oil, Soyabean oil, Castor oil, Labrafil M 2125, Captex 355, Oleic acid, Basil oil, Span 20, Span 80, Kolliphor ELP, Tween 80, Tween 20, Transcutol HP, PEG 200, PEG 400, Propylene Glycol) in separate 5 mL stopper vials, and then mixing them using a vortex mixer. The vials containing the mixture were subsequently stored at a temperature of 25 ± 1.0°C in an isothermal shaker for a period of 72 hours to achieve equilibrium. Once equilibrated, samples were extracted from the shaker and centrifuged at a speed of 3000 rpm for 15 minutes. The resulting supernatant was collected and passed through a 0.45µm membrane filter. The concentration of Tretinoin was then analyzed in the oils, surfactant, and Co-surfactant using UV Spectroscopy within a range of (λmax= 351 nm). 2.4. [8, 9]

Optimization of nano-emulsion formulation

The emulsifying ability of the excipients previously screened for solubility was evaluated for optimization through the formation of a nano-emulsion. This involved conducting a screening procedure followed by a study on the impact of different components [10].

Phase studies

Different trials for ternary phase diagram

The process of phase diagrams entails the representation of three components: surfactant, co-surfactant (Smix), oil, and water, with each component representing a vertex of a triangle. Ternary mixtures with different compositions of these components were created. In every ternary mixture formed, the combined concentrations of surfactants, co-surfactants, and oil always amounted to 100%. The precise quantities of the three components were measured. Subsequently, the mixture was heated gently to a temperature of 45–50ºC and agitated vigorously to achieve a uniform mixture. Distilled water was gradually added to the mixture until a clear solution was obtained. The mass ratios of surfactant and co-surfactant were varied as 1:1, 1:2, and 2:1. Different concentration ratios of oil and the mixture of surfactant and co-surfactant were tested at 1:9, 2:8, 3:7, 4:6, and 5:5. Ternary mixtures were created based on these ratios, and the amount of water required to form a transparent solution was then plotted on the pseudo-ternary phase diagram alongside the other components.

Preparation of nanoemulsion

For the preparation of nanoemulsion, oil and surfactant was added to the Tretinoin and then this

mixture was heated at 50ºC for homogenization of the components. After getting clear solution co-surfactant was added in it. Out of the total mixture 100 mg of sample was withdrawn and then added 5 ml of distilled water; then it was inverted 50-60 times and kept overnight. Selected the formulations from pseudo-ternary phase were subjected to various physical stability tests.

Physical stability of nanoemulsion

Heating-Cooling Cycle: Nanoemulsion was exposed to varied temperatures (4˚C and 45˚C for not more than 48 h) to determine the consequence of temperature variations on its stability.

Centrifugation Study: To detect any creaming, cracking or phase separation Tretinoin Nanoemulsion was centrifuged for 30 mins at 5000 rpm. All measurements were done in triplicate.

Freeze Thaw Cycle: Tretinoin Nanoemulsion was exposed to 3 freeze thaw cycles ranging between −21°C and +25°C with storage at each temperature for not less than 48 h to observe the efficiency of dispersibility.

Characterization of nanoemulsion

Determination of Emulsification, pH and Drug content

Emulsification was done by adding 1 ml of self-emulsifying formulations into a beaker containing 200 ml methanol at 37°C. The sample was stirred and visually monitored to determine the time for complete emulsification. 1 gram of nanoemulsion was dispersed in 100 ml of distilled water and the pH was recorded using a pH meter by bringing it in contact with the gel and allowing it to equilibrate for 1 min. pH was measured in triplicate and average values were calculated.25 mg of the tretinoin was dissolved in 10 mL of methanol, sonicate for 1min. to obtain clear solutions. The resultant solutions were centrifuged 10000 rpm for 30 min. Using methanol as a blank, tretinoin drug content is estimated by analyzing the extract spectrophotometrically at (λmax=351nm) [11].

Evaluation of nanoemulsion

Transmission Electron Microscopy (TEM) Analysis

The TEM analysis of nanoemulsion was performed for morphological characterization and visualization of emulsion droplets. Nanoemulsion formulation was diluted with deionized water and mixed by gentle shaking. A drop of sample obtained after dilution was placed on copper grids, stained with 1% phosphotungstic acid solution for 30s, and finally kept under electron microscope to visualize the particle morphology [12].

Particle size and Zeta Potential

The particle size (PS) and zeta potential of nano emulsionwere determined by the method of photon correlation spectroscopy and electrophoretic mobility, respectively, using a Zeta sizer Nano instrument. Prepared emulsion was diluted 100 times, added into the sample cell, put into the sample holder unit for Zeta potential and particle size determination, respectively [13].

Preparation of gel incorporating Tretinoin-based nano emulsion

The selected surfactant is dissolved in either the aqueous phase or the oil phase. Based on the solubility, the drug is then added and solubilized in the oil phase or aqueous phase followed by heating. Then one phase is gradually added into another with continuous stirring till the temperature of the mixture reaches to room temperature.The appropriate gelling agent is dissolved in distilled water with continuous stirring to prepare gel base. The pH of prepared gel is adjusted, then the nanoemulsion system is incorporated slowly into the prepared gel at a particular ratio with continuous stirring to get nanoemulgel preparation [14].

Table 1: Composition of different formulation tretinoin nanoemulsion gel

Sr. No	Composition	Formulation ode		
		F1	F2	F3
1	Tretinoin nanoemulsion (mL)	5	5	5
2	Carbopol 934 (%w/v)	1%	1.5%	2%
3	NaOH	QS	QS	QS

Characterization of gel

The following parameters were evaluated for optimization of nanoemulsion based gel.

Incorporation of Castor Oil nano-emulsion into gel for Topical drug delivery

The gel of optimized formulation was prepared by dispersing the formulation successfully in Carbopol 934 different concentrations and then subjected for characterization as F1, F2 and F3

Physical Appearance

It was done by using visual observation of all gel formulation.

pH determination

The pH of gel formulations was determined using digital pH meter. One gram of gel was dispersed in 100 ml of distilled water and the pH was recorded using a pH meter by bringing it in contact with the gel and allowing it to equilibrate for 1 min. pH was measured in triplicate and average values were calculated. [15]

Measurement of gel viscosity

Viscometer was used for rheological studies. The sample (30 g) was placed in a beaker and given five minutes to acclimate before the dial reading was measured with a T-4 spindle spinning at five revolutions per minute. The viscometer's matching dial reading was noted at the speed. The dial reading that corresponded to each decrease in spindle speed was recorded. Each measurement was repeated 3 times and the average ± SD is reported [16].

Spreadability Test

A laboratory-made device with two glass slides, the lower slide connected to a wooden plate and the upper slide attached to a balance by a hook, was used to measure the spreadability of gel. The lower slide received 1 gram of gel, whereas the upper slide received weight. When weight was applied, the upper slide moved linearly in the direction of the weight, and the amount of time needed for the upper slide to completely displace was noted. Using the weight required for displacement spreadability was calculated by using Equation 1

$S = m l / t$ ……Eq (1)

S is spreadability, m is the weight tied to the upper slide, l is the length of the glass slide and t is the time taken [17].

Drug content

By combining 100 mg of the gel with 10 mL of methanol, the drug concentration of each batch of Tretinoin nanoemulsion gel formulation was assessed independently. To obtain clear solutions, the resultant solutions were filtered through a filter. Further were centrifuged 10000 rpm for 30 min. Using methanol as a blank, the drug content of these samples was analyzed at (λmax 351 nm) by using UV-spectrophotometer scanned [18].

In vitro drug release

The permeation of Tretinoin bearing nanoemulsion gel through dialysis membrane was performed in Franz-type diffusion cells. The receptor medium was phosphate buffer (pH 6.8) which was constantly stirred at 100 rpm with a small magnetic bar. The receptor compartment was maintained at 37±0.2◦C by a circulating water jacket. An amount of nano emulsion gel equivalent to desired amount of drug was placed in the donor compartment. Samples were withdrawn from the receptor compartment via the sampling part at 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12 and 24 and immediately replaced with an equal volume of fresh receptor solution. Triplicate experiments were conducted for each study and sink conditions were always maintained. All samples were analyzed for Tretinoin content on UV

spectrophotometrically at λ_{max}=351nm of drug **[19-22].**

Drug release kinetic studies

In the present study, raw data obtained from in vitro release studies was analyzed, where in data was fitted to different equations and kinetics model to calculate the percent drug release and release kinetics of optimized formulation. The kinetic models used were a Zero-order equation, First-order, Higuchi's model and Korsmeyer-Peppas equation [23-25].

Zero order kinetics

It can be used to describe the drug dissolution of several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems, as well as matrix tablets with low soluble drugs in coated forms, osmotic systems, etc. In its simplest form, zero order release can be represented as:

Q0 - Qt = K0t

Where, Qt is the amount of drug dissolved in time t, Q0 is the initial amount of drug in the solution (most times, Q0= 0) and K0 is the zero-order release constant expressed in units of concentration/time. To study the release kinetics, data obtained from in vitro drug permeation studies were plotted as cumulative amount of drug released versus time.

Zero-order kinetics

A zero-order release would be predicted by the following equation.

$$\mathbf{A_t = A_0 - K_0 t}$$

Where:

A_t = Drug release at time't'

A_0 = Initial drug concentration

K_0 = Zero order rate constant (hr^{-1})

When the data is plotted as percent drug release versus time, if the plot is linear then the data obeys zero-order release kinetics, with a slope equal to K_0.

First order kinetics

A first-order release would be predicted by the following equation.

$$\mathbf{Log\ C = Log\ C_0 - K_t/2.303}$$

Where:

C = Amount of drug remained at time't'

C_0 = Initial amount of drug

K= First-order rate constant (hr^{-1})

When the data is plotted as log percent drug remaining versus time yields a straight line, indicating that the release follows first-order kinetics. The constant 'K' can be obtained by multiplying 2.303 with slope values.

Higuchi's model

Drug release from the matrix devices by diffusion has been described by following Higuchi's classical diffusion equation:

$$\mathbf{Q = [D\varepsilon/\tau\ (2A - \varepsilon C_s)]\ C_s t^{1/2}}$$

Where:

Q= Amount of drug released at time't'

D= Diffusion coefficient of the drug in the matrix

A = Total amount of drug in unit volume of matrix

C_s = the solubility of drug in the diffusion medium

ε = Porosity of the matrix

τ = Tortuosity

t = Time (hrs.) at which 'Q' amount of drug is released.

Equation may be simplified if one may assume those Ds, Cs and A are constant. Then equation becomes:

Q=K

When the data is plotted according to equation i.e., drug released versus square root of time, yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to 'K'.

Korsmeyer-Peppas Model

The release rates from controlled release polymeric matrices can be described by the equation:

$$\mathbf{Q = K_1 t^n}$$

Where:

Q = Percentage of drug released at time't'

K = Kinetic constant incorporation structural and geometric characteristics and 'n' is the diffusion exponent indicative of the release mechanism.

For Fickian release, n = 0.45 while anomalous (non-Fickian) transport, n ranges between 0.45 and 0.89 and for zero-order release, n = 0.89.

RESULTS AND DISCUSSION

Melting point

The melting point range of Tretinoin was determined to be between 179.3±1.15 and 180.7±1.08°C and Reference melting point is 178-184°C this indicating the absence of impurities in the drug sample. [26].

UV Spectroscopy

Determination of absorption maxima in methanol

A double beam UV-visible spectrophotometer was used for quantitative analysis of the drug. A 7 µg/ml solution of Tretinoin in methanol was scanned in the range of 200-400 nm. The result of UV spectrum of Tretinoin is shown in Figure 1

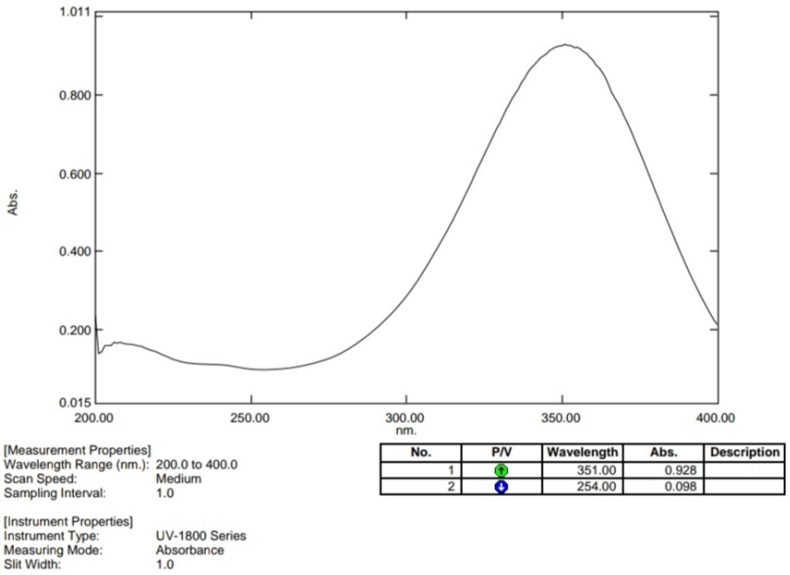

Figure 1: UV Spectrum of tretinoin in methanol

Table 2: Absorption maxima (λ_{max}) of tretinoin in methanol

Name of drug	Absorption maxima (λ_{max})	
	Observed	Reference
Tretinoin	351	351

The maximum wavelength of Tretinoin was observed at 351 nm similar to literature (Table 2) [26].

Preparation of standard curve of Tretinoin in methanol

Table 3: Calibration curve of tretinoin in methanol (λ_{max}= 351 nm)

Sr. No.	Concentration. (µg/ml)	Mean±SD
1	1	0.158± 0.0005
2	2	0.296± 0.001
3	3	0.414± 0.003
4	4	0.527± 0.002
5	5	0.653± 0.002
6	6	0.785± 0.002
7	7	0.928± 0.0005

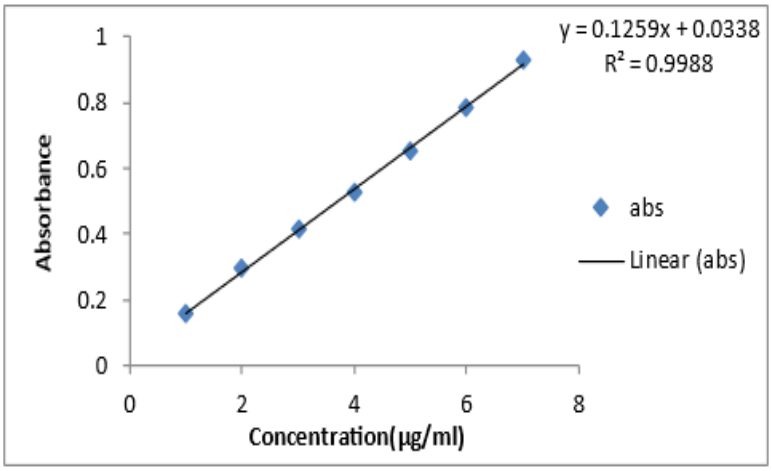

Figure 2: Standard calibration curve of tretinoin in methanol

Table 4: Result of regression analysis of UV method

Statistical parameters	Results
λ_{max}	351nm
Regression equation (y =mx+ c)	y = 0.1259x + 0.0338
Slope (m)	0.1259
Intercept (C)	0.0338
Correlation coefficient (R^2)	0.9988

The calibration curve for Tretinoin was obtained by using the 1 to 7 µg/ml concentration of Tretinoin in methanol. The absorbance was measured at 351 nm. The calibration curve of Tretinoin as shows in graph indicated the regression equation y = 0.1259x + 0.0338and R^2 value 0.9988, which shows good linearity as shown in Table 4 and Figure 2.

Solubility of drug in solvents, Oils, Surfactant, Co-surfactant

Table 5: Solubility of drug in solvents, Oils, Surfactant, Co-surfactant

S.no.	Oil/Surfactant/co-surfactant	Solubility (mg/ml)
1.	Castor oil (Oil)	5.469±0.165
2.	Oleic acid (Oil)	3.324±0.012
3.	Captex 355 (Oil)	2.532±0.016
4.	Olive oil (Oil)	1.14±0.012
5.	Labrafil M2125 (Oil)	0.549±0.021
6.	Soyabean oil (Oil)	0.472±0.012
7.	Basil oil (Oil)	0.02±0.012
8.	Span 20 (Surfactant)	0.651±0.0012
9.	Tween 20 (Surfactant)	0.364±0.0016
10.	Span 80 (Surfactant)	0.198±0.0015
11.	Kolliphor ELP (Surfactant)	0.097±0.0012
12.	Tween 80 (Surfactant)	0.057±0.0016
13.	Transcutol HP (Co-Surfactant)	0.747±0.0012
14.	Propylene glycol (Surfactant)	0.47±0.0016
15.	PEG 200 (Surfactant)	0.507±0.0016
16.	PEG 400 (Surfactant)	1.683±0.21

Table 5 Demonstrate that Tretinoin exhibits maximum solubility in Castor oil, Oleic acid, and Captex among the tested oils. Similarly, among various surfactants, Tretinoin shows the highest solubility in Span 20, Tween 20, and Span 80. For different co-surfactants, Tretinoin achieves the greatest solubility in PEG 400 and Transcutol HP.

Phase studies

Phase studies of castor oil+ Tween 20 + PEG400 [1:1, 1:2, 2:1]

While conducting the screening studies it was observed that 2 combinations involving three different Co-surfactant and surfactant which have same oil combination have % transmittance every close as shown in **Table 6** Thus, it became necessary to finalize the final combination by construction of pseudo-ternary phase diagram which will indicate the highest region for formation of nanoemulsion will be the final

combinations elected having fixed Smix ratio O:
Smix ratio as 1:1 and 2:1.

Table 6: Combination of Castor oil + Tween 20+ PEG 400 (Surfactant: Cosurfactant (1:1), (1:2), (2:1).

Formulation code	Ratio	Castor oil(mg)	Tween20 (mg)	PEG 400 (mg)	Transmittance (%) (λ=630 nm)	Appearance (up to 100ml water)
	1:1					
B1A1	1:9	97.5	438.75	438.75	63.2±0.1	Turbid
B1A2	2:8	195	390	390	52.4±0.15	Turbid
B1A3	3:7	292.5	341.25	341.25	50.4±0.15	Turbid
B1A4	4:6	390	292.5	292.5	68.3±0.1	Transparent
B1A5	5:5	487.5	243.75	243.75	63.1±0.1	Turbid
	1:2					
B1A6	1:9	97.5	292.5	585	64.2±0.1	Transparent
B1A7	2:8	195	260	520	60.2±0.20	Turbid
B1A8	3:7	292.5	227.5	455	59.5±0.1	Turbid
B1A9	4:6	390	195	390	56.4±0.20	Turbid
B1A10	5:5	487.5	162.5	325	51.5±0.36	Turbid
	2:1					
B1A11	1:9	97.5	585	292.5	58.2±0.1	Turbid
B1A12	2:8	195	520	260	55.5±0.1	Turbid
B1A13	3:7	292.5	455	227.5	57.2±0.1	Turbid
B1A14	4:6	390	390	195	61.5±0.32	Turbid
B1A15	5:5	487.5	325	162.5	60.8±0.1	Turbid

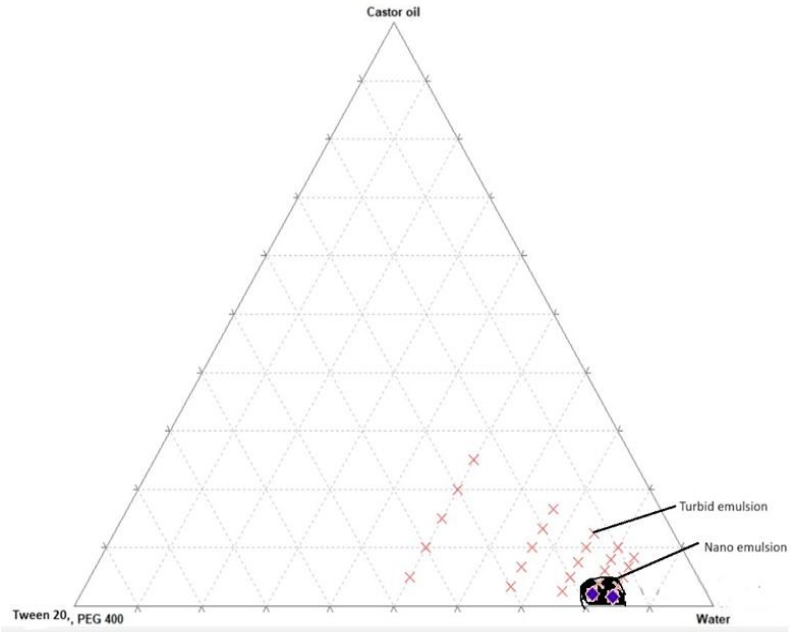

Figure 3: Castor oil, Tween 20, PEG 400

Discussion: From the Figure 3it can be concluded that the formation of nanoemulsion depend on the ratio's in which the oil, surfactant and co-surfactant are added. The pseudo-ternary diagram depicts the region (shade as black) where there is the probability of formation of Nano emulsion from the **Figure 3**and the red crossed region indicates the turbidity of the emulsion and the blue dots has been assigned to the highly transparent emulsion.

Phase studies of castor oil+ Tween 20 + Transcutol HP [1:1, 1:2, 2:1]

While conducting the screening studies it was observed that 2 combinations involving three different Co-surfactant and surfactant which have same oil combination have % transmittance every close as shown in **Table 7.** Thus, it became necessary to finalize the final combination by construction of pseudo-ternary phase diagram which will indicate the highest region for formation of nanoemulsion will be the final combinations elected having fixed Smix ratio O: Smix ratio as 1:1 and 2:1.

Table 7: Combination of castor oil+ Tween 20 + Transcutol HP [1:1, 1:2, 2:1]

Formulation code	Ratio	Castor oil(mg)	Tween20 (mg)	Transcutol HP (mg)	Formulation code	Transmittance (%) (λ=630 nm)
	1:1					
B2A1	1:9	97.5	438.75	438.75	84.1±0.1	Turbid
B2A2	2:8	195	390	390	87.2±0.1	Transparent
B2A3	3:7	292.5	341.25	341.25	82.5±0.25	Turbid
B2A4	4:6	390	292.5	292.5	68.3±0.15	Turbid
B2A5	5:5	487.5	243.75	243.75	63.1±0.1	Turbid
	1:2					
B2A6	1:9	97.5	292.5	585	85.4±0.32	Turbid
B2A7	2:8	195	260	520	70.3±0.25	Turbid
B2A8	3:7	292.5	227.5	455	71.2±0.15	Turbid
B2A9	4:6	390	195	390	72.5±0.25	Turbid
B2A10	5:5	487.5	162.5	325	72.3±0.2	Turbid
	2:1					
B2A11	1:9	97.5	585	292.5	93.3±0.1	Transparent
B2A12	2:8	195	520	260	71.1±0.1	Transparent
B2A13	3:7	292.5	455	227.5	87.3±0.15	Turbid
B2A14	4:6	390	390	195	84.4±0.26	Turbid
B2A15	5:5	487.5	325	162.5	82.4±0.245	Turbid

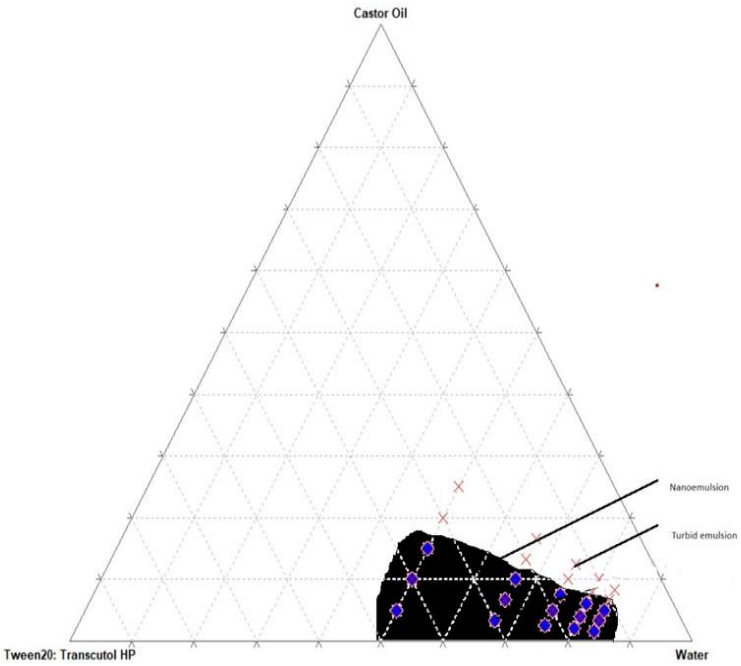

Figure:4 castor oil+ Tween 20 + Transcutol HP

Discussion: From the **Figure 4**it can be concluded that the formation of nanoemulsion depend on the ratio's in which the oil, surfactant and co-surfactant are added. The pseudo-ternary diagram depicts the region (shade as black) where there is the probability of formation of nanoemulsion from the **Figure 4**and the red crossed region indicates the turbidity of the emulsion and the blue dots has been assigned to the highly transparent emulsion. **The selective formulations were B1A4, B1A6, B2A2, B2A11 and B2A12 which were renamed as E1, E2, E3, F4 and E5.**

Table 8: Composition of Optimized formulations by pseudo ternary diagram

Sr. No.	Formulation Code	Oil(mg)	Surfactant Tween20(mg)	Co-surfactant PEG400 (mg)	Co-surfactant Transcutol HP (mg)	Water (ml)
1	E1	50	150	150	-	5
2	E2	50	225	450	-	5
3	E3	50	200	-	200	5
4	E4	50	450	-	225	5
5	E5	50	400	-	200	5

Physical stability of nano-emulsion [27, 28]

Table 9: Physical stability test of formulations (E1 and E2 castor oil+ Tween 20 + PEG400) (E3-E5 castor oil+ Tween 20 + Transcutol HP)

Formulation Code	Physical stability test			Inference
	Heating	Freeze thaw cycle	Centrifugation	
E1	Stable	Phase separation	Disperse	Passed
E2	Stable	Phase separation	Disperse	Passed
E3	Stable	Phase separation	Disperse	Passed
E4	Stable	Phase separation	Disperse	Passed
E5	Stable	Phase separation	Disperse	Passed

Formulations E1 to E5 are the transparent nano-emulsions possesses all the physical stability parameters.

The characterization studies of nanoemulsions were done by analyzing Appearance, pH, Transmittance and Drug content.

Characterization of nano-emulsion [29]

Table 10: Characterization of different nanoemulsion formulations

Formulation Code	Emulsification	Visual appearance	pH	% Transmittance	%Drug content
E1	Rapid	Clear	5.21±.061	96.20±0.20	79.59±0.48
E2	Rapid	Clear	5.350±0.407	92.667±0.252	81.92±0.66
E3	Rapid	Clear	5.513±0.116	95.333±0.306	85.63±0.66
E4	Rapid	Clear	5.730±0.173	99.30±0.2	91.67±1.11
E5	Rapid	Clear	5.09±0.053	98.167±0.153	89.65±0.84

From the table 10.It became clear that there is not much difference in formulation because transmittance as a prime importance parameter was selected as the final formulation. Drug content of E4 formulation has been found 91.67±1.11 which is the highest among all.

Evaluation of nano-emulsion [30-1]

TEM

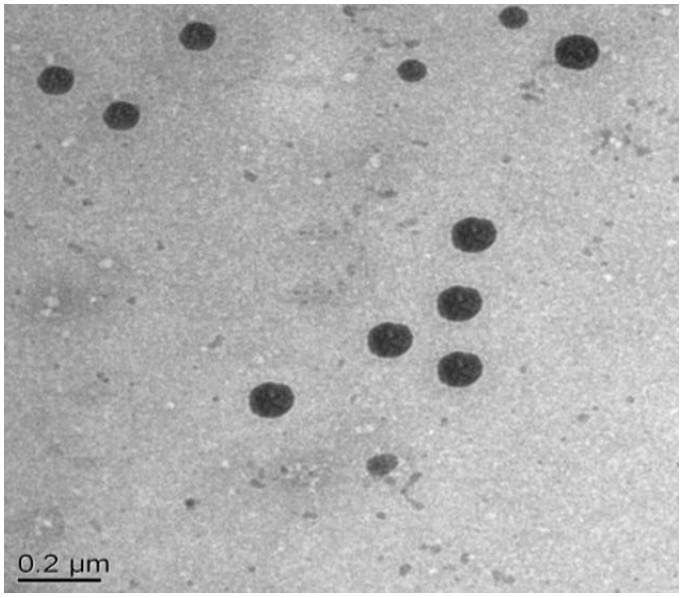

Figure 5: TEM of nanoemulsion

From the figure 5 it was concluded that the prepared nanoemulsion of the optimized formulation (E4) was found to be spherical to oval in shape.

Particle Size

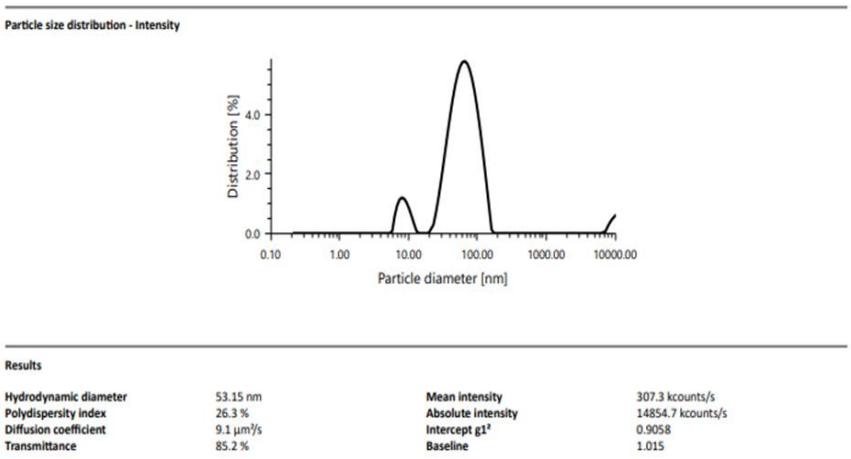

Figure: 6: Particle size of nanoemulsion (E4)

From the figure 6 it was concluded that particle size 53.15 nm. Thus, the results show that the particle size of the formed nanoemulsion was in required range therefore a transparent nano-emulsion was analysed to determine whether it is in the nano-emulsion i.e. less than 100nm.

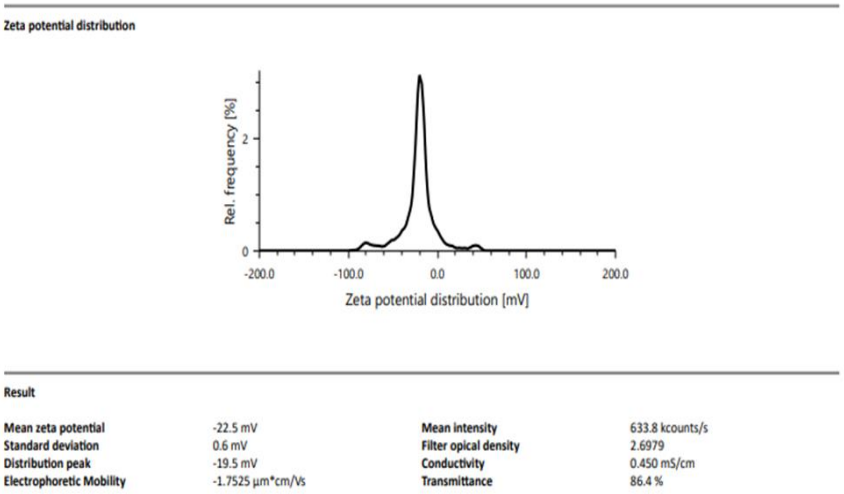

Figure 7: Zeta potential graph of nanoemulsion (E4)

Figure 7 demonstrated zeta potential of E4 formulation was -22.5 mV represents stability of nanoemulsion.

Incorporation of Castor Oil nano-emulsion into gel for Topical drug delivery

The gel of optimized E4 formulation was prepared by dispersing the formulation successfully in Carbopol 934 different concentrations and then subjected for characterization.

Evaluation of Tretinoin nanoemulgel

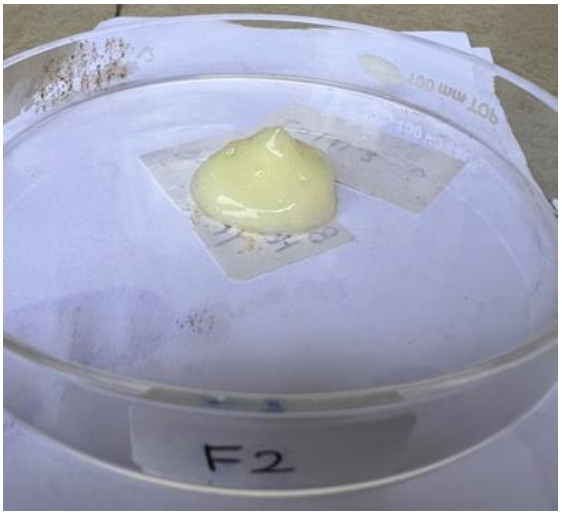

Figure 8: Visual Appearance of nano-emulsion gel

Table 11: Visual Appearance of nano-emulsion gel

Sr. no.	Formulation code	Visual appearance
1	F1	Gel not formed
2	F2	Smooth gel without lump formation
3	F3	Smooth gel without lump formation

The prepared gel were examined visually for their consistency and found to smooth appearance. Out of three developed gel formulations batches F2 and F3were showed good homogeneity with absence of lumps. So those batches were used in further study.

Table 12: Evaluation of tretinoin nanoemulgel

Sr. no.	Formulation code	pH (Mean ± S.D)	Viscosity (Mean ± S.D)	Spreadability (g.cm/sec) (mean ± SD)	% Drug Content
1	F2	5.15±0.030	28.6±0.25	1026±11.5	98.76±0.917
2	F3	5.10±0.002	27.96±0.15	1038±5.85	94.84±0.485

According to Table 12, the pH of all formulations ranged from 5.96±0.12 to 6.03±0.03. The viscosity (Visco QC100) of the formulations ranged between 28.6±0.25 and 27.96±0.15, and the spreadability ranged from 1026±11.5 to 1038±5.85. The drug content in the gels was between 98.76±0.917 and 94.84±0.485, demonstrating satisfactory drug content percentages for all formulations, thereby confirming the suitability of the adopted method for gel formulations.

Fourier Transform Infrared Spectroscopy (FTIR) Study

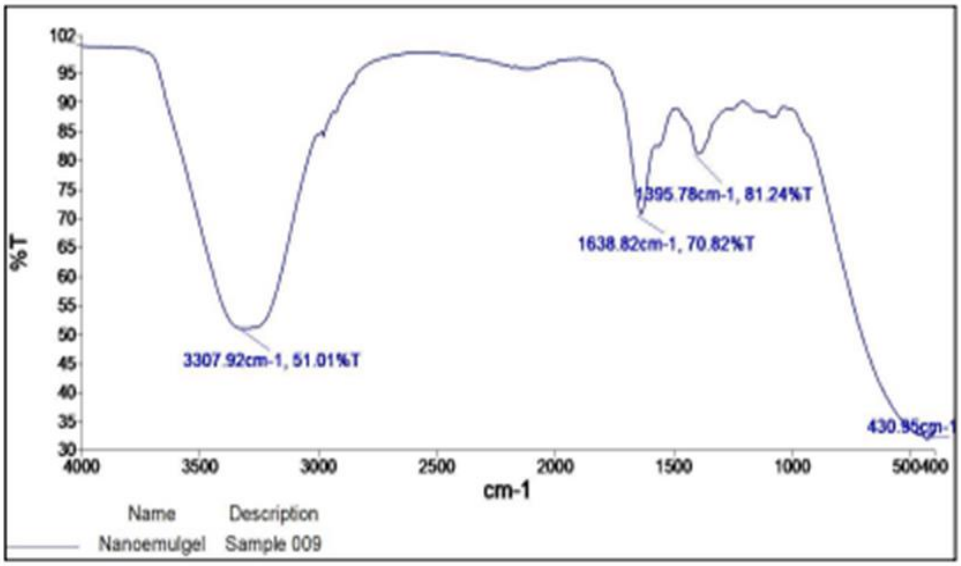

Figure 9: FTIR Spectra of formulation F2

The FT-IR spectra of final formulation (F2) indicate that characteristic peak of tretinoin was not visible in the nanoemulgel spectra which indicates that oil was completely encapsulate in the nano-emulsion gel.

In vitro drug release studies

Table 13: In vitro drug release of tretinoin loaded nano-emulgel & control gel formulation (λmax= 351 nm)

Sr. No.	Time (hr)	Drug release of control gel formulation (%)	Drug release of Formulation F2 (%)
1	0	0	0
2	0.25	26.43±0.64	12.77±0.64
3	0.5	45.28±0.97	15.63±0.84
4	1	60.43±0.84	23.15±0.66
5	1.5	83.52±0.49	30.35±0.49
6	2	92.09±0.80	37.13±0.49

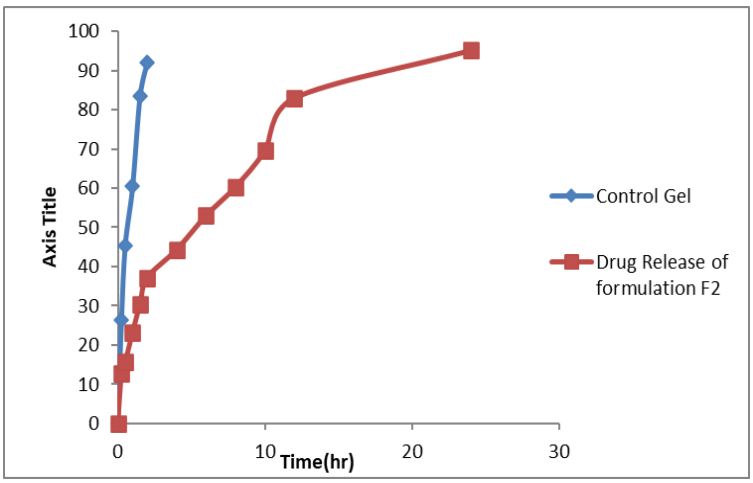

Figure 10: Percentage drug release of Tretinoin loaded nano-emulgel & control gel formulation

The in-vitro drug release of Tretinoin loaded nano-emulgel & control gel formulation was given in a Table 13.and figure 10. The release from control gel shows 92.09±0.80 within 2hrs. On the other hand, the release of drug in gel formulations F2G2 showed 95.17±0.66% in 24hrs in the phosphate

buffer salinewith in 24hr. Drug loaded gel formulation F2 (containing 1.5% Carbopol 934) demonstrated maximum drug release up to 81.200±0.267%, within 24hr check absorbance in UV spectrophotometer range at (λmax= 351 nm).

Drug release kinetic studies

Zero order kinetics

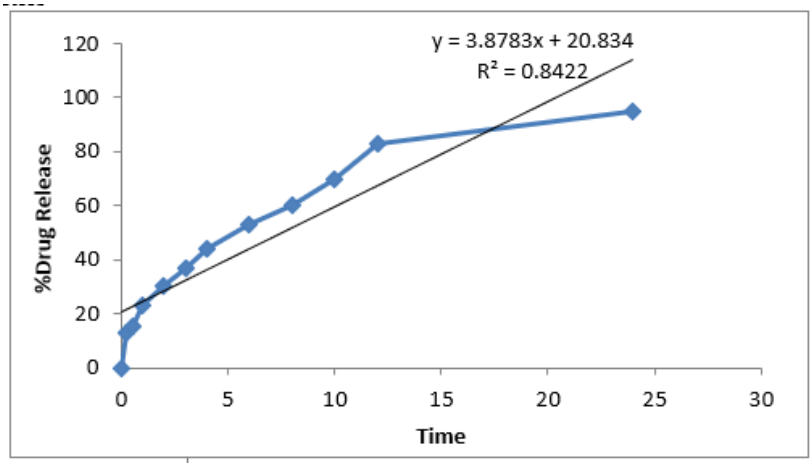

Figure 11: Zero order graph of formulation F2G2

First Order Kinetics

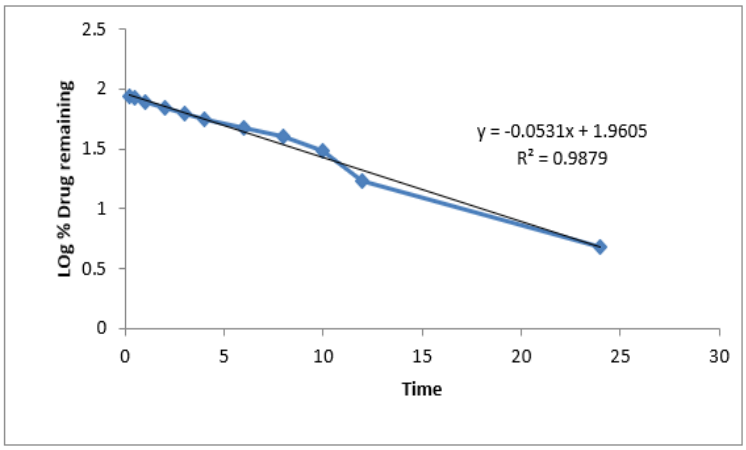

Figure 12: First order graph of formulation F2G2

Higuchi's Model

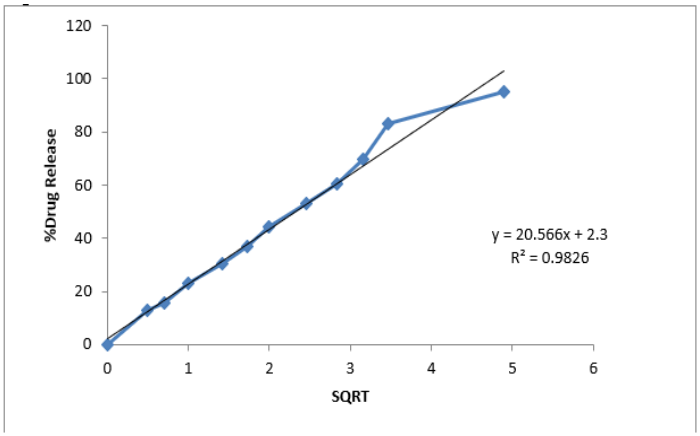

Figure 13: Higuchi order graph of formulation F2G2

Korsmeyer-Peppas Model

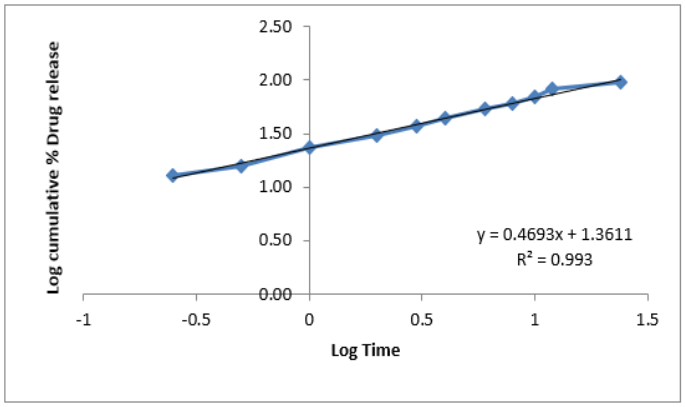

Figure 14: Korsmeyer-peppas order graph of formulation F2G2

Table 14: Kinetic equation parameter of formulation F2G2

Formulation Code	Zero-order		First-order		Higuchi		K. Peppas	
	K_0	R^2	K_0	R^2	K_0	R^2	K_0	R^2
F2	3.878	0.842	-0.0531	0.987	20.566	0.982	0.469	0.993

Mathematical models are commonly used to predict the release mechanism and compare release profile. For the optimized formulation, the % drug release vs time (zero order), log percent drug remaining vs time (first order), log per cent drug release vs square root of time (Higuchi plot), and log of log % drug release vs. log time (Korsmeyer and Peppas Exponential Equation) were plotted. In each case, R^2 value was calculated from thegraph and reported in Table 14 and Figure 11 to Figure 14. Considering the determination coefficients, K. Peppas was found (R^2=0.993) to fit the release data best. It could be concluded from the results that the drug was released from tretinoin loaded nanoemulgel by a sustain mechanism.

CONCLUSION

In this investigation, a tretinoin micro emulsion was used to enhance residence time, bioavailability, and onset of action. Physicochemical evaluation determined tretinoin melting point. UV spectrophotometer analysis showed its absorption maxima. Tretinoin had maximum solubility in certain oils, surfactants, and co-surfactants. FTIR spectroscopy revealed no drug-excipient interaction. Tretinoin nanoemulsion was formulated. Nanoemulsion E4 was chosen for topical gel preparation. The pH of gel F2 with 1.5% carbopol was determined. Spreadibility studies were done. We concluded that nanoemulsion design and preparation method can sustain drug release. The study developed drug delivery systems for sustained release of tretinoin.

Authors Contributions

The experimental design, guidance, supervision, and review work for the research was carried out by Mr. Narendra Singh Solanki. On the other hand, Sheela Suthar performed the experimental work, developed and optimized the formulations, interpreted the results, and wrote this manuscript. Both authors have read and approved the final manuscript.

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HOW TO CITE: Sheela Suthar*, Narendra Singh Solanki, Formulation and Evaluation of Tretinoin Nanoemulgel to Enhance Its Activity, Int. J. of Pharm. Sci., 2025, Vol 3, Issue 3, 1113-1131. https://doi.org/10.5281/zenodo.15016645