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Research article

Preparation and characterization of a fluocinolone acetonide nanoemulsion as a topical delivery system

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ABSTRACT

The objective of current research project is to characterize and formulate nano-emulsion of Fluocinolone-acetonide as an efficacious treatment for atopic dermatitis. Fluocinolone-acetonide is a synthetic hydrocortisone derivative used in dermatology to reduce inflammation of skin. It categorize under BCS class II i.e. low solubility and high penetrability. Solubility is low and absorption rate is very minimal. Consequently, to overcome these issues nano-emulsions have been formulated. Topical nano-emulsion containing 0.01% Fluocinolone-acetonide with several oils (castor oil, olive oil, almond oil and oleic acid), surfactant (Tween 20, Tween 80), co-surfactant (PEG 400, PEG 200) and distilled water. Several water-in-oil nano-emulsion were formulated by ultra-sonication method. Nano-emulsion formulations were characterized for thermodynamic stability test, pH, viscosity, FTIR and in-vitro drug release study and stability studies.

Keywords: nanoemulsion, atopic dermatitis, solubility, absorption, fluocinolone acetonide

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INTRODUCTION

Nano-emulsions are isotropic bi-phasic dispersion of two immiscible liquids which stabilize with the help of amphiphilic surfactant. Nano-emulsions have characteristics that make them more effective transport system than macro-emulsions as they do not show the problem of agglomeration and sedimentation which are generally related with macro-emulsions. Nano-emulsions system considered as a promising vehicle in topical treatment of different skin diseases due to their fast permeation through skin lipids. They are harmless and non-irritant formulation that can be used for skin or membranes. They are able to formulate a variety of lipophilic drugs and lipophobic drugs in order to enhance the accumulation of drug at the targeted site of action. Nano-emulsion enhances drug retention time at the target site, hence causes less side effects. Fluocinolone-acetonide is a synthetic hydrocortisone derivative and corticosteroid that classified under BCS class II i.e. less solubility and high permeability. It has been used broadly in dermatological formulations. Atopic dermatitis is very common chronic, inflammatory skin disease which occurs repeatedly and mainly found in children. It is also known as eczema. Only 50% of the patients suffering with this disease are having increased immunoglobulin E (IgE) and allergic sensitization.

Advantages

- Enhanced bioavailability and increased drug loading capacity.
- It shows reproducible plasma drug profile.
- It enhances the efficacy of drug by minimizing the dose.
- It can provide controlled and sustained release of entrapped drug [1, 2].

Objectives

- The major aim is to formulate transdermal nano-emulsion.
- Select suitable excipients based on physic-chemical characteristics of drug.
- Characterization and formulation of nano-emulsion
- Increasing the solubility of drug.
- Enhancing the therapeutic effect at the site of action.

MATERIALS AND METHODS

Material

Fluocinolone-acetonide was purchased from Dermo care Laboratories, Gujarat, Oleic acid Tween 20 and PEG 400 was procured from Meerut Institute of Engineering and Technology, Meerut India.

Pre-Formulations Studies

Melting point determination

Capillary method was a technique by which melting point of drug is measured. One end of tube is sealed and drug is filled up to the height of 3 mm. Melting point was determined with the help of melting point apparatus.

Solubility Studies

Solubility was determined by adding the drug in the solvent till saturation was achieved at room temperature and placed for a day.

Through UV spectrophotometer supernatant liquid was analyzed.

Identification and compatibility study by ftir

The acquired drug was uniformly mixed with Potassium bromide and sample was filled in dye and put into Potassium bromide press, then pellet was taken out and placed in IR. The drug sample was examined between the ranges of 4000-600 cm^{-1}.

For the compatibility studies of the drug and excipients a pre-concentrate of drug, oil and Smix was formed, with the help of capillary tube one drop of mixture placed on readymade KBr pellet and placed in IR and scan between the ranges of 4000-600 cm^{-1}. The spectra of the pre-concentrate mixture were compared with the spectra of pure oil, drug, surfactant/ co-surfactant mixture.

Identification and calibration of drug by UV spectrophotometer

The drug sample (100 mg) was weighed on weighing balance and was dissolved in 100 ml ethanol taken in 100 ml volumetric flask. It was marked as primary stock solution of drug i.e. 100 µg/ml. Five different aliquots from primary stock solution was taken in volumetric flask and make-up the volume with ethanol to get concentration of 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 50 µg/ml. The resulting solution was measured by UV spectrophotometer at 238 nm using ethanol as blank, the entire study is performed thrice [3, 4, 5].

Total phenol determination

Parkhe et al [11] developed a technique for determining total phenolic content. 1 ml Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) sodium carbonate were combined with 2 ml extracts or standards. The mixture was allowed to sit at room temperature for 15 minutes. A UV/visible spectrophotometer were used to measure the colour generated at 765 nm. The total phenolic content was determined using the gallic acid standard graph, and the findings were represented in milligram per 100 milligrams of gallic acid.

Total flavonoids determination

Parkhe et al [11] developed a technique for determining total flavonoid content. 1 mL of 2% $AlCl_3$ methanolic solution was added to 3 mL of each extract or standard and allowed to stand at room temperature for 15 minutes; the absorbance of the reaction mixture was measured at 420 nm with a UV/visible spectrophotometer. The flavonoid content was determined using a standard graph of quercetin and represented as quercetin equivalent (mg/100mg).

Preparation of nano-emulsion

Pseudo-ternary phase diagram study

To determine the phase behavior of the selected ternary components (Smix, oil and aqueous phase), several ternary phase diagrams were prepared. Phase diagrams were constructed using aqueous dilution technique at different Smix levels (1:1, 1:1.5, 1.5:1, 1:2 and 2:1). To construct a phase diagram at Smix ratio 1:1 several weight combination oil (11, 22, 33, 45 ml….) and Smix (20, 40, 60, 80, 100 ml…) were taken in different beakers of 10 ml capacity. The content of each beaker was sonicated the resulting mixture was triturated against distilled water taken in 25 ml burette and titration is done until the clarity of resulting mixture was disappear which indicates the end point titration. The percentage (in w/w) of each consumed component used in titration was calculated and therefore plotted to get ternary phase diagram with the help of software [5, 6].

Table 1: Formulation of nano-emulsion

Formulation code	Oil (ml)	Smix (ml)	Amount of drug (mg)	Fixed amount of water	Total weight
Smix = 1:1 (Tween 20: PEG 400), Oil phase (Oleic acid)					
F1	29.9	96.1	17.64	14	140
Smix= 1.5:1 (Tween 20: PEG 400), Oil phase (Oleic acid)					
F2	29.9	96.1	17.64	14	140
Smix= 2:1 (Tween 20: PEG 400), Oil phase (Oleic acid)					
F3	29.9	96.1	17.64	14	140
Smix= 1.5:1 (PEG 400: Tween 20), Oil phase (Oleic acid)					
F4	29.9	96.1	17.64	14	140
Smix= 2:1 (PEG 400: Tween 20), Oil phase (Oleic acid)					
F5	29.9	96.1	17.64	14	140

Characterization of nanoemulsion

Thermodynamic stability

Thermodynamic stability test was performed on selected formulation.

Heating cooling cycle

In this study the refrigerator temperature was set between 4˚C and 45˚C to perform 6 heating-cooling cycles with storage at each temperature for 48 hrs. Centrifugation procedure was perform on formulations which shows better stability at these temperatures.

Centrifugation

Those formulations that were stable during heating cooling cycle are subjected to centrifugation for 30 minutes at 5000 rpm by using centrifuge. The formulation in which phase separation does not occur were recommended to further studies.

Determination of pH

pH meter was used for the determination of several nano-emulsion formulation. To avoid error measurement of reading of formulation done in triplicate.

Viscosity determination

Brookfield viscometer was used for the measurement of viscosity of nano-emulsion formulations. 100 ml of nano-emulsion was taken in 150 ml beaker and rheology was determined by utilizing 61 spindle number at 10, 20, 40 and 50 rpm.

In-Vitro diffusion studies

Franz diffusion cell was used to determine the diffusion studies of the prepared nano-emulsion. Cellophane membrane was placed on receptor compartment and 5 ml of nano-emulsion was taken in donor compartment of the cell and diffusion studies were performed at 37±1˚C using 25 ml of phosphate buffer (pH 7.4) as the dissolution medium. 2 ml of nano-emulsion was taken out at specific time interval at and each sample was replaced with equal amount of fresh media to maintain sink conditions. Through UV spectrophotometer samples are examined at 238 nm.

Droplet size distribution

Analysis of the droplet size was carried out by the Malvern Zeta Sizer Nano series. 5ml of sample was filtered through nylon membrane followed by sonication and then transferred into cuvette of zetasizer and then scanning was done for 10 minutes.

Refractive index determination

For the determination of refractive index Atago Refractometer was used. From nano-emulsion one drop was placed on the lens of refractometer and temperature was adjusted between 20˚ to 25˚ C and readings were taken in triplicate to avoid error.

Determination of electrical conductivity

Digital conductivity meter was used to determine the electrical conductivity of nanoemulsion. This test help to determine whether the sample was water continuous and oil continuous.

Transmission electron microscopy

The structural and morphological behavior of the nano-emulsion is determined by the TEM i.e. Model Morgani 268D operated at 70kV. A small drop of sample which is in concentrated form is placed over a wax coated paper and film made of copper is applied externally, staining is done with the help of 2% w/v uranyl acetate [7, 8].

RESULTS AND DISCUSSION

The current research was done to formulate nano-emulsion of Fluocinolone acetonide for efficacious treatment of atopic dermatitis. Five nano-emulsion formulations are prepared and evaluated for several parameters.

Determination of melting point

Melting point of the Fluocinolone-acetonide was found to be 265˚C

Drug solubility

Table 1: Solubility of Fluocinolone-acetonide in oils, surfactant and co-surfactant

Oils	Surfactant	Co-surfactant			
Liquid paraffin	5mg/ml	Tween 20	28mg/ml	Polyethylene Glycol 400	45mg/ml
Castor oil	15mg/ml	Tween 80	35mg/ml	Polyethylene Glycol 200	32mg/ml
Oleic acid	40mg/ml				
Olive oil	10mg/ml				

Identification and calibration of drug by UV spectrophotometer

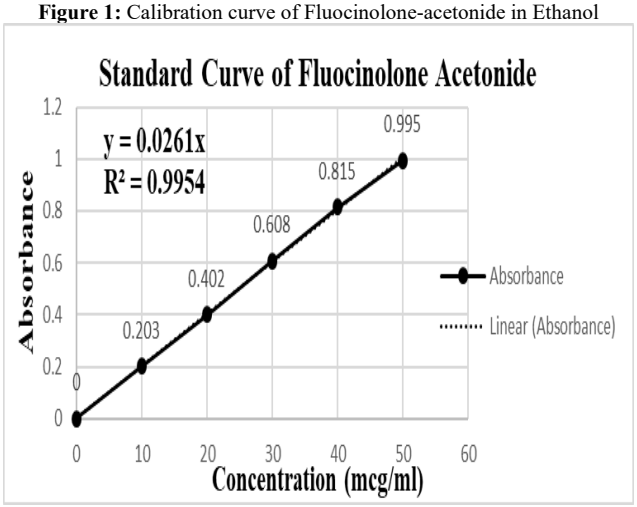
Standard calibration plot of Fluocinolone acetonide in ethanol

Table 2: Calibration curve of Fluocinolone-acetonide in ethanol

S. No.	Concentration (µg/ml)	Absorbance (nm)
1	0	0
2	10	0.203± 0.004
3	20	0.402 ± 0.006
4	30	0.608 ± 0.007
5	40	0.815 ± 0.006
6	50	0.995 ± 0.009
	Slope	**0.020048571**
	Intercept	**0.00261**

UV spectrophotometer shows that Fluocinolone acetonide gives maximum absorption at 238 nm.

Figure 1: Calibration curve of Fluocinolone-acetonide in Ethanol



FTIR spectroscopy

Figure 2: FTIR spectrum of Fluocinolone-acetonide (Drug)

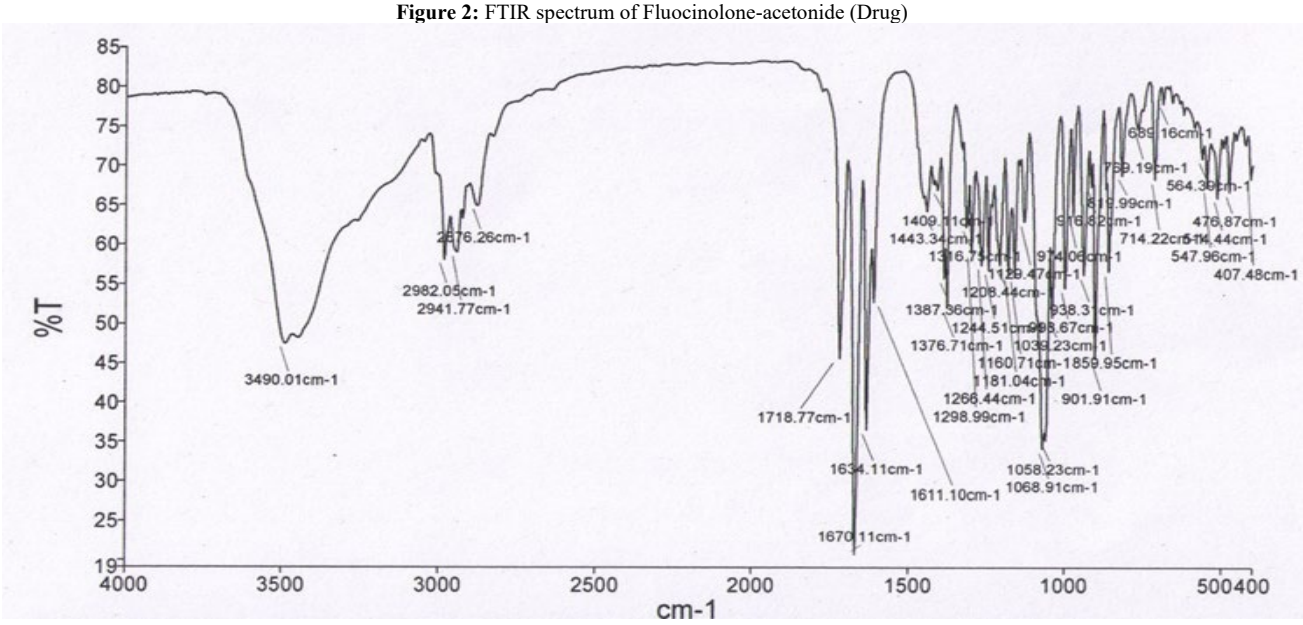


Figure 3: FTIR spectrum of Fluocinolone-acetonide + Oleic Acid + S-mix (Tween-20: PEG 400)

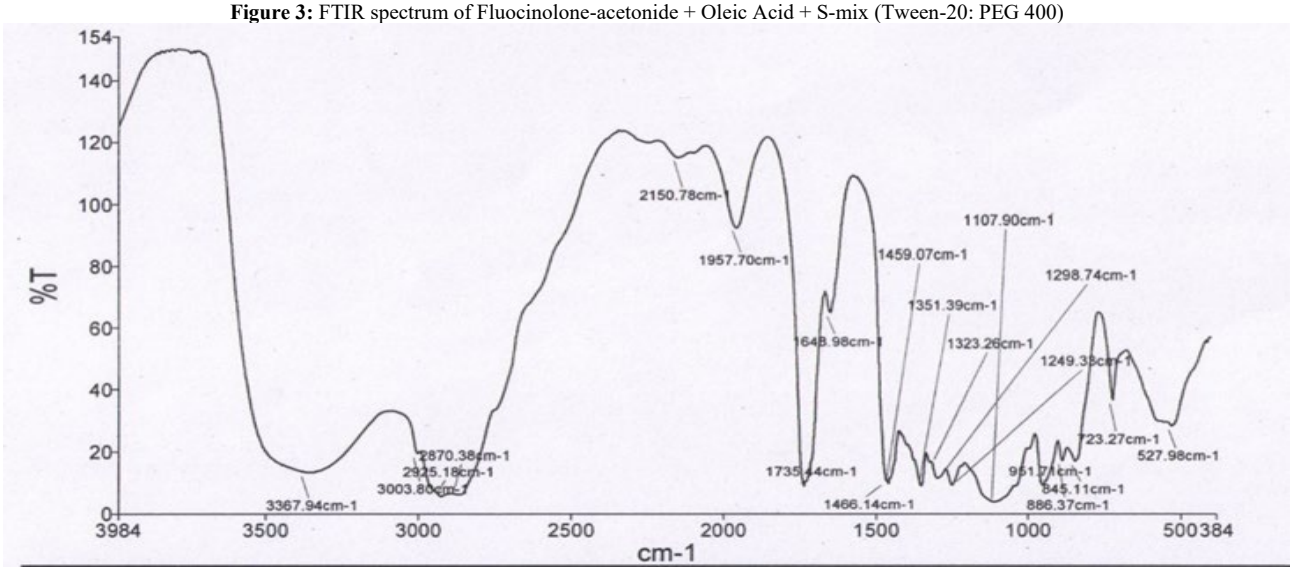
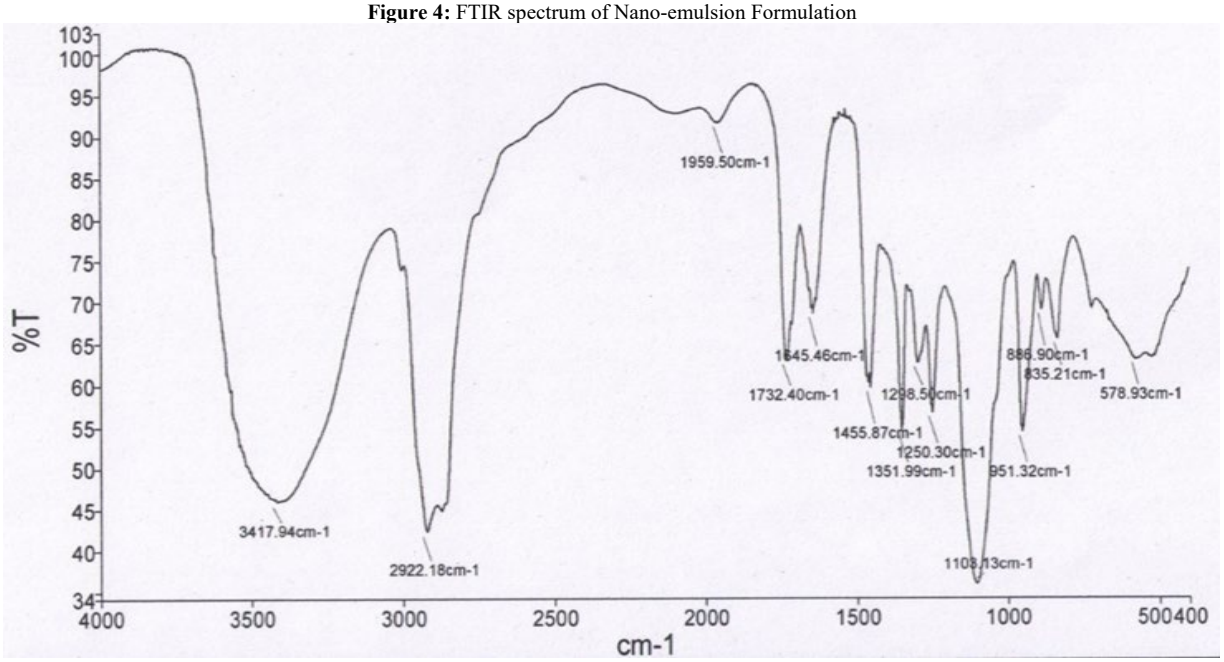
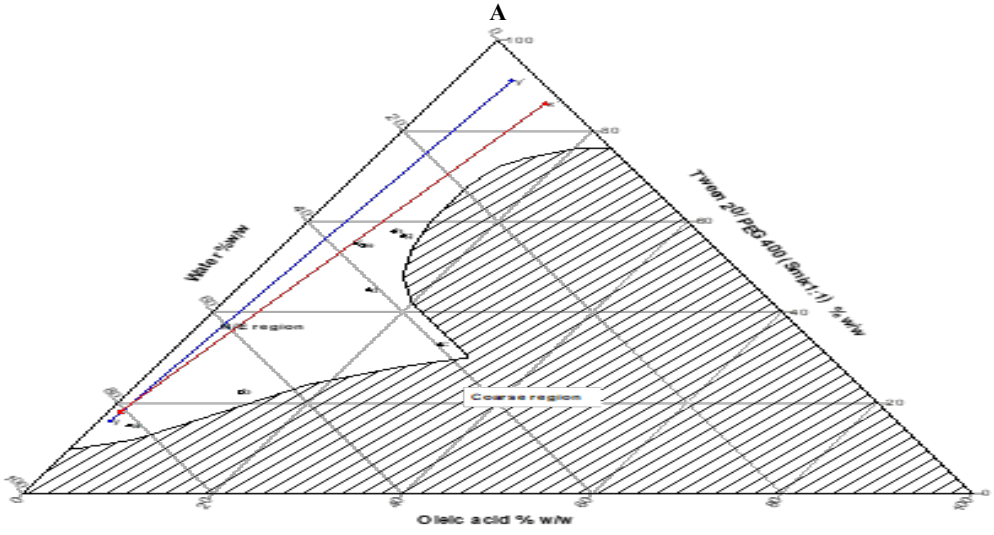


Figure 4: FTIR spectrum of Nano-emulsion Formulation

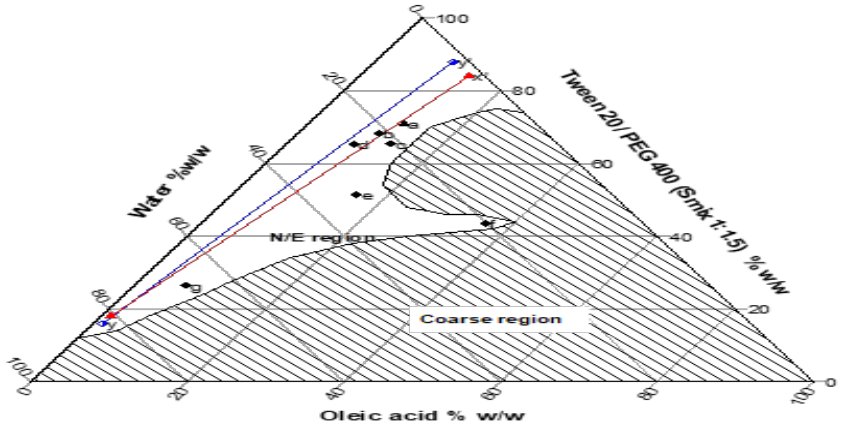


Ternary phase diagrams

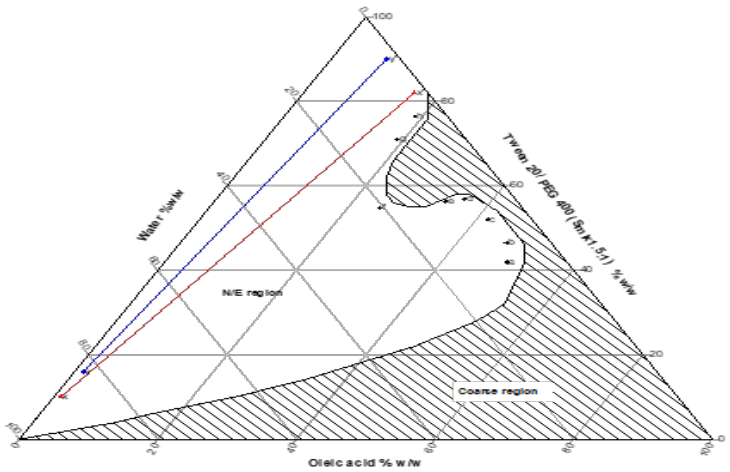
Figure 5: A, B, C, D and E represents ternary phase diagram of nano-emulsion formulation



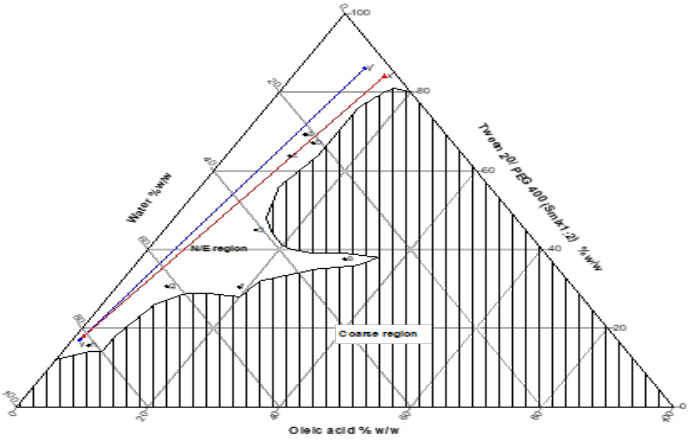
B


Water %w/w
Tween120/ PEG 400 (Smix 1:1.5) % w/w
N/E region
Coarse region
Oleic acid % w/w

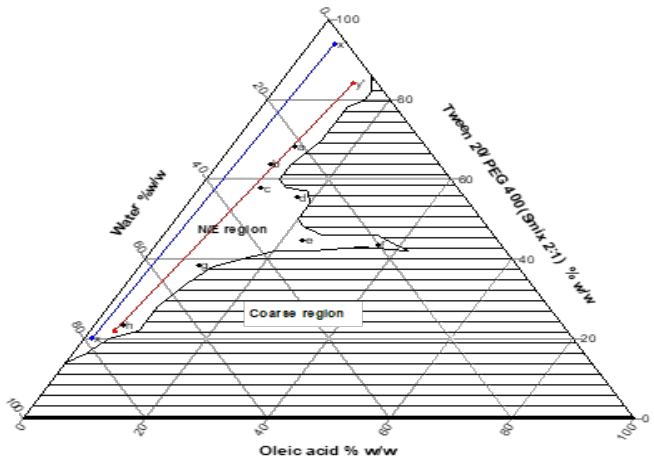
C


Water %w/w
Tween 20/ PEG 400 (Smix1:1.5) % w/w
N/E region
Coarse region
Oleic acid % w/w

D


Water %w/w
Tween 20/ PEG 400 (Smix1:2) % w/w
N/E region
Coarse region
Oleic acid % w/w

E


Water %w/w
Tween 20/ PEG 400 (Smix 2:1) % w/w
N/E region
Coarse region
Oleic acid % w/w

Droplet size distribution

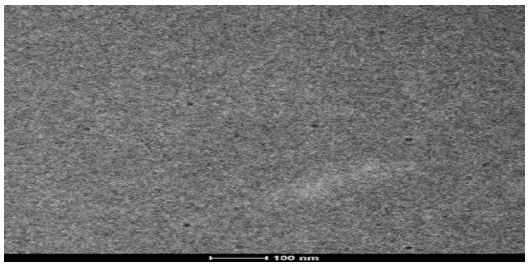
Table 3: Droplet size and PDI of the formulation

Formulation code	Droplet size (nm)	PDI
F1	62.84	0.751
F2	25.57	0.474
F3	25.23	0.975
F4	119.9	1.000
F5	18.75	0.499

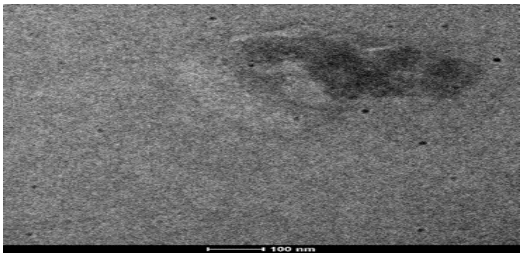
Transmission electron microscopy

Figure 6: A and B represents morphology of nano-emulsion formulation

A



B



From the above images, it was concluded that particle size of nano-emulsion is lies within 100nm.

Thermodynamic stability studies

Table 4: Thermodynamic stability studies

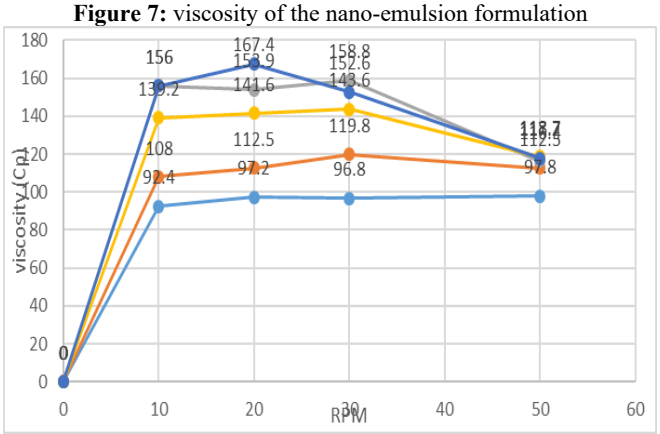
Formulation	Centrifugation	Heating-Cooling Cycle
F1	Phase separation is not observed	Stable
F2	Phase separation is not observed	Stable
F3	Phase separation is not observed	Stable
F4	Phase separation is not observed	Stable
F5	Phase separation is not observed	Stable

Viscosity study

Table 5: viscosity determination

Rpm	F1	F2	F3	F4	F5
0	0	0	0	0	0
10	156	139.2	156	108	92.4
20	167.4	141.6	153.9	112.5	97.2
40	152.6	143.6	158.8	119.8	96.8
50	117.7	118.7	116.4	112.5	97.8

Figure 7: viscosity of the nano-emulsion formulation



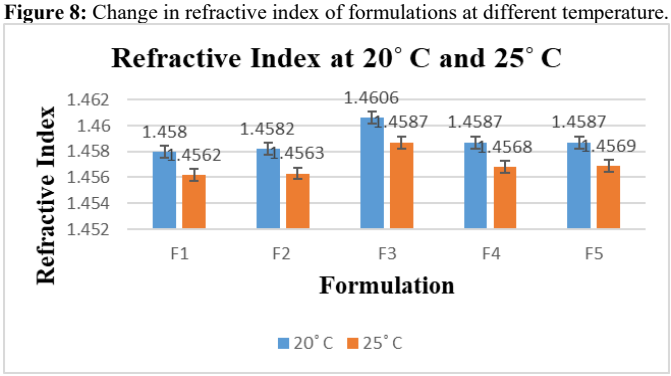
Viscosity of all the formulations was found to be in the range of 92.4 to 167.4 cp which is higher than the viscosity of water, which states that system was water in oil.

Refractive index

Table 6: Refractive index of formulations.

S. No	Batch	20˚C	25˚C
1	F1	1.4580	1.4562
2	F2	1.4582	1.4563
3	F3	1.4606	1.4587
4	F4	1.4587	1.4568
5	F5	1.4587	1.4569

Figure 8: Change in refractive index of formulations at different temperature.



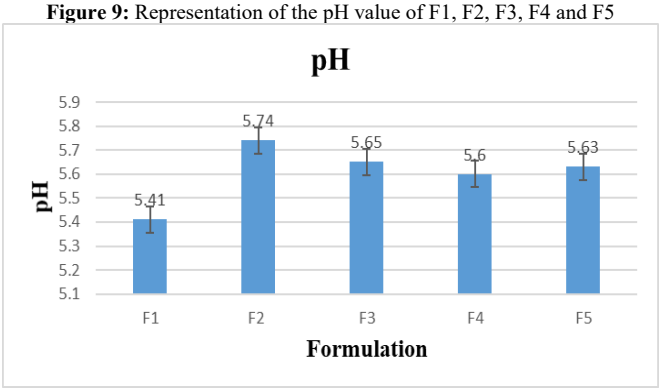
Refractive index of all the formulations was found to be in the range of 1.4580 to 1.4606 which is higher than the RI of Milli Q water, which states that system was water in oil.

pH Determination

Table 7: pH value of the formulations

Formulation code	pH value
F1	5.41
F2	5.74
F3	5.65
F4	5.60
F5	5.63

Figure 9: Representation of the pH value of F1, F2, F3, F4 and F5



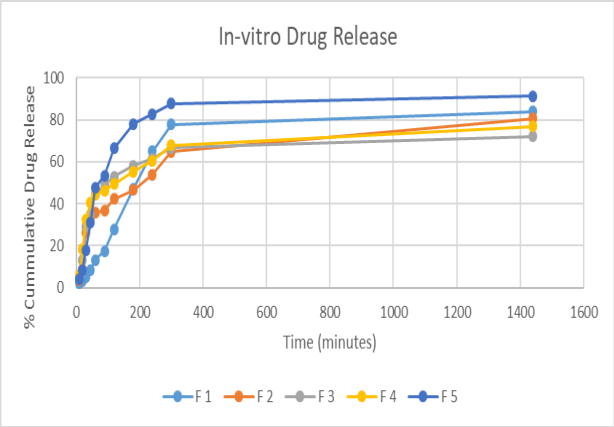
pH of all the formulations as found to be in the range of 5.41 to 5.74 which was nearby to the pH of skin.

In-Vitro drug release studies

Table 8: In-vitro drug release studies

Time(min)	F1	F2	F3	F4	F5
10	2.23	3.66	4.14	5.81	3.9
20	3.19	8.9	12.96	18.2	8.43
30	4.85	26.31	28.93	32.27	17.73
45	8.19	31.79	34.65	40.38	30.84
60	13.2	35.6	44.66	44.19	47.52
90	17.49	36.8	48.96	46.33	53.49
120	27.94	42.28	53.01	49.43	66.39
180	47.29	46.57	58.02	55.39	78.04
240	64.93	53.96	61.35	60.4	82.8
300	77.8	64.69	66.6	67.79	87.81
1440	84	80.66	72.08	76.85	91.15

Figure 10: In-vitro drug release of nano-emulsion formulation



The outcome of in-vitro drug release studies from various nano-emulsion formulations are shown in table 8 and graphically data is represent in figure 10. The nano-emulsion formulation batch F5 exhibits the best release drug profile when compared to the other nano-emulsion formulation F1, F2, F3 and F4.

CONCLUSIONS

This research concluded that Fluocinolone-acetonide is incorporated into nano-emulsion formulation by ultra-sonication technique for the improvement of solubility and absorption. Nano-emulsion is more desirable for the transdermal drug administration because it enhanced absorption rate through skin. Nano-emulsions have characteristics that make them more effective transport system than macro-emulsions as they do not show the problem of agglomeration and sedimentation which are generally associated with macro-emulsions. On the basis of ternary phase diagram and drug solubility test Tween-20, Oleic acid and Polyethylene Glycol 400 was selected and these are eminently used as an appropriate transporting system for introducing Fluocinolone-acetonide for transdermal drug delivery. Five formulations were formulated as per the composition of nano-emulsion. The developed nanoemulsion formulation coded as F1, F2, F3, F4 and F5. Several evaluation parameters such as droplet size distribution, TEM, thermodynamic stability test, pH, viscosity, electrical conductivity and refractive index indicates that F5 is more appropriate as compared to F1 to F4. On the basis of in-vitro skin permeation study F5 formulation shows more drug release i.e. 91.15% as compared to F1 to F4 also the size of F5 formulation was found to be 18.75nm. From above studies it was concluded and examined that Fluocinolone-acetonide nano-emulsion is efficacious for transdermal application in the treatment of atopic dermatitis although in-vivo studies are further needs to be conducted to authenticate the results in a more specific way.

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