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Original Article

THE CHOICE OF COMPONENTS AND THE IMPACT OF SURFACTANTS AND CO-SURFACTANTS ON EPLERENONE NANOEMULSION SYNTHESIS FOR GEL-BASED TRANSDERMAL APPLICATION

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ABSTRACT

Objective: This research aims to establish an efficient methodology for selecting nanoemulsion components to synthesize eplerenone nanoemulsion for gel-based transdermal applications.

Methods: The chemical compatibility study of eplerenone was investigated by FTIR, DSC, and solubility in oils, surfactants, and co-surfactants as the criteria for choice. We used visual appraisal and grading to assess the effectiveness of emulsification. Various excipients were tested depending on solubility. The final appearance, dispersibility, and ease of emulsification were used to visually assess the degree of self-emulsification of oil and emulsifier in a 1:3 mass ratio. Co-surfactants were assessed by mixing particular emulsifiers in a 2:1 (w/w) ratio with co-surfactants, and the oily component was added at a 1:3 (w/w) ratio to evaluate Smix's emulsification potential. A central composite design synthesized, evaluated, and optimized eplerenone nanoemulsions. Optimized nanoemulsions were characterized after a thermodynamic stability study for droplet size, ζ potential, viscosity, refractive index, pH measurements, and TEM. All the selected formulations were found to be stable, and the droplet size was found to be<110 nm.

Results: Eplerenone was chemically compatible, and its maximum solubility was 171.3±0.92 and 169.3±2.22 in Kollicream®OA and Paceol, respectively. The evidence impressively found that Tween 20 and Kolliphor®EL were discovered as active emulsifiers, and Transcutol®P was revealed to be a co-surfactant. Outcomes showed that the emulsification efficacy of Kolliphor®EL (3% w/w) was able to emulsify Kollicream®OA (1% w/w), and Paceol failed. As well, Smix [Kolliphor®EL (2% w/w) and Transcutol®P (1% w/w)] were able to emulsify Kollicream®OA (1% w/w).

Conclusion: The main conclusion from this work is the application of a visual appraisal and grading system to assess the final appearance, dispersibility, and ease of emulsification to eradicate the toxicity and irritation that nanoemulsions can cause. Optimised nanoemulsions can further formulate eplerenone's nanoemulsion gel for transdermal application.

Keywords: Smix, Oil phase, Nanoemulsion region, Pseudo ternary phase diagram, Emulsification efficacy

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INTRODUCTION

Droplet sizes of emulsions of 20-500 nm are denoted as Nanoemulsions; they are also rarely called tiny emulsions [1]. Nanoemulsions are known by various names, such as submicron, ultrafine, and mini-emulsions [2–5]. A nanoemulsion system operates with two isotropically dispersed, non-mixable liquids and can be used with this technique. Nanoemulsion typically consists of either oil spread in water or reverse. However, they can also form nanometer-sized water-in-oil or oil-in-water droplets. W/O emulsions were utilised less often than O/W nanoemulsions [6]. For delivering nutrients or lipophilic drugs, they are a fantastic alternative [1, 7]. Nanoemulsions are stable and appear transparent or slightly transparent when viewed without magnification. They also do not settle down or cream. It has been discovered that they eliminate absorption variability and enhance biological availability and absorption [8]. Nanoemulsions are used widely due to their low viscosity, translucency, and comparatively high kinetic stability [9]. Typically, surfactants and co-surfactants are required to sustain the stability of emulsions and extend their expiry date. These are the molecules that come together at the boundary between oil and water to keep the mixture stable, using various mechanisms like electrostatic repulsive interaction and steric stabilisation [5, 10].

Reviewing nanoemulsions used in percutaneous delivery of drugs, most studies on selecting the proper surfactants and co-surfactants have yet to prove to be very successful. An organised screening system was the primary objective of this study, which was to choose oils, emulsifiers, and co-emulsifiers suitable for the synthesis and gel inclusion of the nanoemulsion formulation.

Hence, eplerenone, a lipophilic drug with BCS class II (Log P=1.34), is selected for the study [11, 12]. Surfactants are part of the nanoemulsions, and applying them to the skin's surface typically raises membrane permeability, enabling transdermal flux. Studies have demonstrated that nanoemulsions can regulate the release and increase many medications' biological availability [13, 14]. Nanoemulsions can cause toxicity and irritation issues because they need high concentrations of surfactants to soften [15]. Since it is a skilful drug delivery technique, transdermal drug delivery could benefit from an improved formula with the desired aspects. For this reason, nanoemulsions exhibit superior transdermal permeation as they have high solubilisation capacity for both water-soluble and hydrophobic drugs [15, 16].

The present study aimed to determine the necessity of selecting surfactants carefully and determining the ideal concentration. A visual appraisal system was employed for emulsification efficacy. One of the primary objectives of the investigation was to decide how mass ratios of emulsifiers and co-emulsifiers influence the creation of nanoemulsions in precise ternary phase areas.

MATERIALS AND METHODS

Components

Eplerenone was purchased from Yarrow Chem Products, Mumbai. IMCD India Private Ltd., Bandra (East), Mumbai provided gift samples of Polyoxyl 40 Hydrogenated Castor Oil (Kolliphor®RH 40), Oleyl alcohol (Kollicream®OA), Macrogolglycerol Ricinoleate (Kolliphor®EL), and Macrogol (Kollisolv®PEG400). Glyceryl mono-oleate (Paceol), Oleoyl polyoxyl-6 glycerides (Labrafil®M 1944),

Diethylene glycol monoethyl ether (Transcutol®P), Propylene glycol monolaurate (Lauroglycol™FCC) were gifted by Gattefossé India, Vikhroli (East), Mumbai. Ethanediol, Tween 20, Ethanol, Isopropyl alcohol, Iso Propyl Myristate, PEG, PEG 200, PEG 400, and propylene glycol were procured from Thermo Fisher Scientific India Pvt. Ltd., Mumbai. All remaining chemical compounds and dissolving agents were of analytical grade. The chemicals and excipients were all used exactly as supplied. All remaining chemical compounds and dissolving agents were of analytical grade. The chemicals and excipients were all used exactly as supplied. When necessary, recently made distilled water and buffers were utilised and passed through a 0.45 µm membrane filter. (Deccan Plastics, Pvt. Ltd., Chh. Sambhajinagar, India).

Calibration curve of eplerenone

Dissolving 10 mg of eplerenone in 100 ml of methyl alcohol and a few minor modifications resulted in a concentration of 100 µg/ml solution that served as the stock solution. Using a micropipette, aliquots of this stock solution were transferred into 10 ml volumetric flasks to create a series of samples ranging from 0.2 ml to 2 ml in increments of 0.2 ml, totaling ten readings. Each volumetric flask was then diluted to a final volume of 10 ml using methyl alcohol. The resulting dilutions of 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 µg/ml were analysed using a UV-visible spectrophotometer at a predetermined wavelength (λmax) of 241 nm. A calibration curve was constructed by plotting the concentration of each sample against its corresponding absorbance. This experimental procedure was repeated three times to ensure the accuracy and reproducibility of the results [17, 18].

Preformulation studies

Drug's chemical compatibility: excipients

5 ml rubber-stopped glass vials containing 500 mg of each chosen excipient and 100 mg of eplerenone were carefully weighed and mixed. For 14 days, blends were kept in sealed vials in humidity-controlled ovens set to 60 °C and 40 °C/75 % RH. The same conditions were used to store a standard eplerenone sample that had not been combined with excipients. After 14 d, FTIR and DSC analyses were performed on duplicate samples of drug-excipient combinations [19-21].

Studies on solubility

Eplerenone's solubility was measured in several oils, emulsifiers, and co-emulsifiers and was evaluated using the shake flask technique. Excess eplerenone was combined with 2 ml of methyl alcohol in rubber stopper vials with a capacity of 5 ml. The mixture underwent incubation for 48 h at 37 °C in an orbital shaker incubator (Remi Instruments, India). Subsequently, the blend was centrifuged at 5000 rpm for 10 min to remove any residual particles, and the supernatants were filtered using a 0.45 µm membrane filter (Deccan Plastics, Pvt. Ltd., Chh. Sambhajinagar, India) attached to a syringe. The dissolved eplerenone in the filtrates was measured using a UV spectrophotometer set to a wavelength of 241 nm. Each experiment was conducted in triplicate to ensure the reliability and consistency of the results [22].

Testing of surfactants and co-surfactants: evaluation of the scattering characteristics

Initially, the optical inspection was utilised to assess the emulsification properties. A homogeneous mixture was prepared by precisely measuring and blending a designated quantity of oil and emulsifier in a 1:3 mass ratio. The mixture was heated to 40–50 °C and vigorously mixed to ensure uniformity. Later, 500 mg of the oil-surfactant blend was placed in a 10 ml beaker and slowly mixed using a magnetic blender (1MLH, Remi Equipment Ltd., Mumbai) until dissolved. Gradually, up to 10 ml of water was added, and the degree of self-emulsification was visually evaluated based on the final appearance, dispersibility, and ease of emulsification, as detailed in table 2. Various co-surfactants were assessed by combining specific emulsifiers with co-surfactants in a 2:1 (w/w) ratio. The oily component was integrated into the mixture at a ratio of 1:3 (w/w), vortexed thoroughly, and gently heated to ensure a homogeneous blend. This methodological approach was employed to assess the emulsification capabilities of the co-surfactants [19].

Table 1: Physicochemical properties of eplerenone

Property	Value reported	Value observed[a]	References
Molecular weight	414.4 g/mol ($C_{24}H_{30}O_6$)	--------	
Log P	1.34	1.446±0.0349	[11,12,23]
Melting Point	244 °C	246.03±1.145 °C	
Solubility in water	0.00903 mg/ml	0.008103±0.0009 mg/ml	
Solubility in Methanol	23.2 µg/ml	23.31±0.845 µg/ml	

[[a]Data are expressed as mean±SD, n = 3]

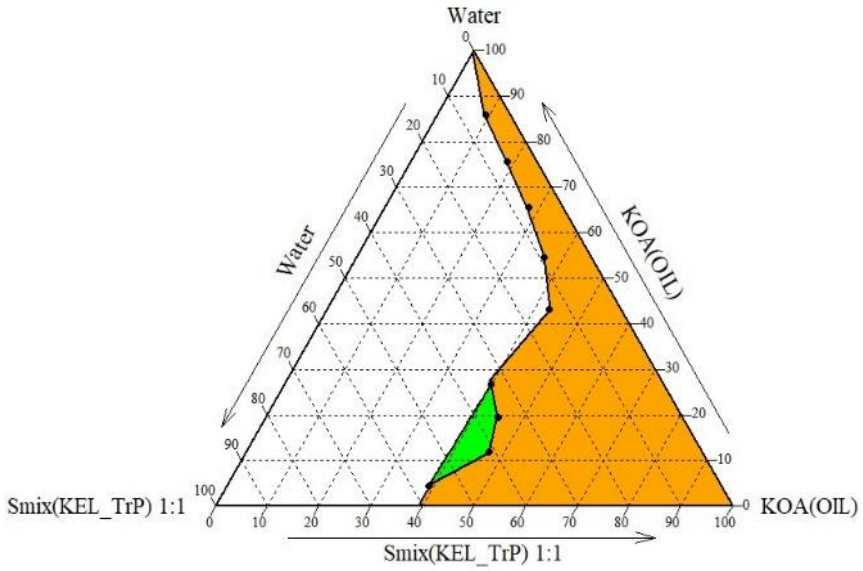
Table 2: Evaluation of emulsification efficacy visually

Dispersibility and presence	Self-emulsification period (min)	Score	Reference
In water, quickly disperse to create a clear, transparent nanoemulsion.	<1	+++(very good)	
In water, droplets of the mixture disperse to form a turbid emulsion.	3–5	+(good)	[19]
The combination produces clusters of oil droplets that do not spread in water.	Not emulsified	-(Poor)	

Development and assessment of eplerenone nanoemulsion

Fabrication of pseudo ternary phase diagram

Chemix school application version 10 was utilised to create ternary phase diagrams. This research was done with ternary combinations that varied in the proportions of oil, emulsifiers, and co-emulsifiers. The ability of Kolliphor®EL to self-emulsify played a role in the surfactant's selection. The oil phase comprised Paceol and Kollicream®OA, while Transcutol®P served as a co-surfactant. Later on, Paceol's inability to emulsify with different surfactants led to its rejection. Pseudo-ternary phase diagrams were created using Smix, a combination of surfactants and co-surfactants, and distilled water. The water titration method was employed in its creation. Different surfactant ratios to co-surfactant mass (1:1, 1:2, 1:3, 3:1, and 3:2) were determined based on the growing surfactant concentration connected with co-surfactant and vice versa. A specific mixing ratio was followed to blend the oil in a separate 10 ml borosilicate glass beaker with a weight ratio of 1:9–9:1. Forty-five different proportions of Smix to oil (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1) were used to create specially created phase boundaries in each phase diagram. Water was measured, and the titration stopped when the different o/w nanoemulsions became clear or slightly bluish. The oil and Smix combinations were gradually diluted in the water phase while translucency was observed. We disposed of the remaining nanoemulsions. Since the sum was 100%, the mass percentages of water, oil, and Smix were recorded at these endpoints. Phase diagrams showing the physical state of nanoemulsions discovered the grouping of the Smix, oil, and aqueous phases in a single region. Each diagram described the nanoemulsion area, with a larger region indicating more efficient emulsification [24, 25].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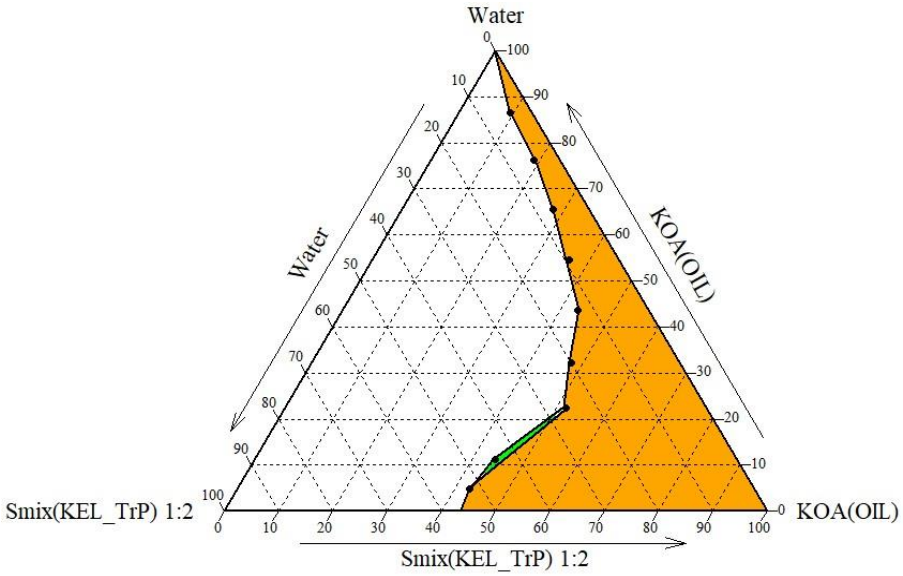
Water
KOA(OIL)
Smix(KEL_TrP) 1:1
KOA(OIL)
Smix(KEL_TrP) 1:1

A (1:1)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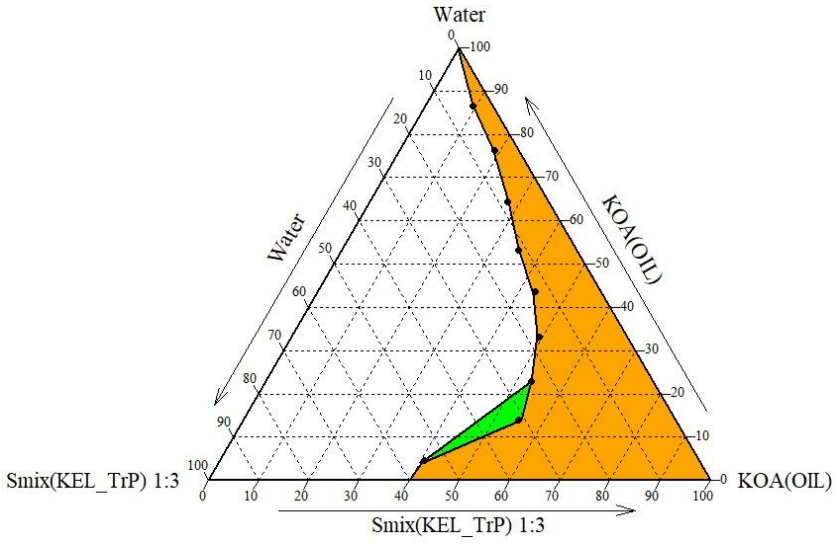
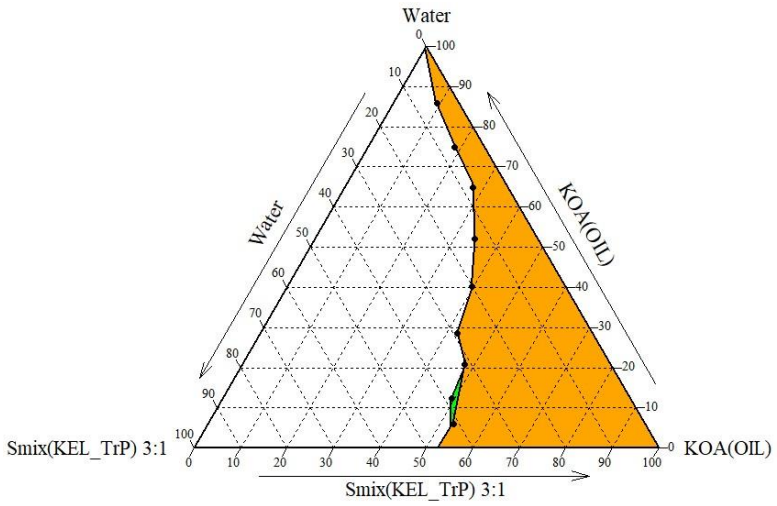
Water
KOA(OIL)
Smix(KEL_TrP) 1:2
KOA(OIL)
Smix(KEL_TrP) 1:2

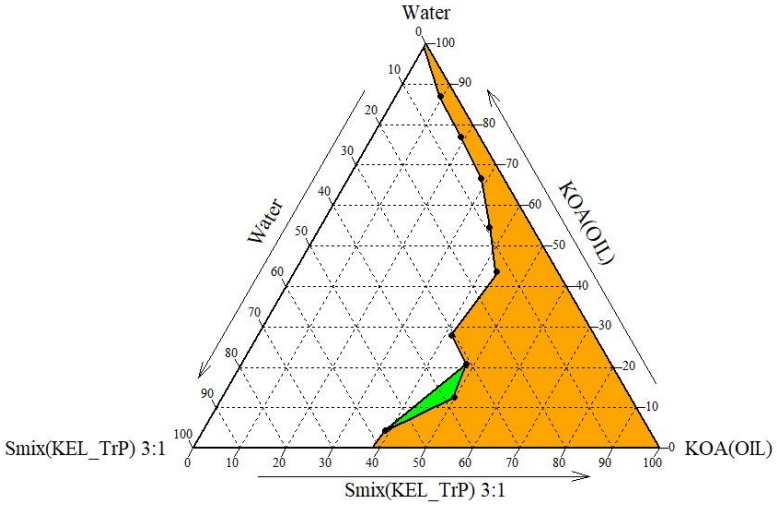
B (1:2)


Water
Water
KOA(OIL)
Smix(KEL_TrP) 1:3
KOA(OIL)
Smix(KEL_TrP) 1:3

C (1:3)



D (3:1)



E (3:2)

Fig. 1: Phase diagrams of (A) Kollicream®OA-Kolliphor®EL-Transcutol®P (1:1) (B) Kollicream®OA-Kolliphor®EL-Transcutol®P (1:2) (C) Kollicream®OA-Kolliphor®EL-Transcutol®P (1:3) (D) Kollicream®OA-Kolliphor®EL-Transcutol®P (3:1) (E) Kollicream®OA-Kolliphor®EL-Transcutol®P (3:2)

Synthesis of eplerenone nanoemulsions

By analyzing pseudo-ternary phase diagrams with the maximum nanoemulsion area, the system loads eplerenone. In this case, oil Kollicream®OA and Smix (1:1 and 1:3; Kolliphor®EL/Transcutol®P) were used. Oil was mixed with a weighed amount of eplerenone in a 10 ml borosilicate beaker and heated to a temperature of 40 to 45 °C in a bath sonicator cleaner (Enertech Pvt. Ltd., Mumbai, India) with gentle stirring. After the addition of Smix, the mixture was titrated against a predetermined volume of water and mixed with a magnetic blender (1 MLH Magnetic Stirrer, Remi Instruments, Mumbai, India) at 500 rpm to produce a coarse emulsion. Probe sonication was then employed from 20 S to 80 S to reduce globule sizes, resulting in clear nanoemulsions (PCI Analytics Pvt. Ltd., Thane, Mumbai) [26–28].

Table 3: Composition of eplerenone nanoemulsions

Ratio of Smix	Formulation code	% w/w components in formulation		
		Oil (%)	Water (%)	Smix (S+CoS %)
NEn-A	E1	5	54.3	40.7
Smix ratio 1:1	E2	10	45.8	44.2
	E3	15	39	46
	E4	20	35.9	44.1
	E5	25	34.6	40.4
NEn-C	F1	5	52.8	42.2
Smix ratio 1: 3	F2	10	44.8	45.2
	F3	15	35.5	49.4
	F4	20	27.6	52.4

Fig. 2: Eplerenone nanoemulsions

Optimization of eplerenone nanoemulsion by CCD (Design Expert®Software Trial Version) 2 factors

Using Design Expert®Software (Trial version 13, Stat-Ease Inc., Minneapolis, USA), we examined three response variables: particle size (Y1) in nm, drug content (Y2) in percent, and PDI (Y3), with two independent variables: Smix concentration (X1) and Ultrasonication Time (X2). This optimisation was applied to nanoemulsion batch E4. Thirteen experimental runs were conducted randomly, consisting of 5 centre points, 4 axial points, and 4 factorial points.

Tables 4 and 5 provide specific details on the collected data [29].

Table 4: Variables used in central composite design

Variables	Levels			
	-α	-1	+1	+α
Independent variables				
X1: Smix (%)	37.9289	40	50	52.0711
X2: Ultrasonication time (S)	20	20	80	71.2132
Dependent variables				
Y1: Particle size (nm)	Minimize			
Y2: Drug content (%)	Maximize			
Y3: PDI	Minimize			

Table 5: Optimization of eplerenone nanoemulsion by central composite design

Run	Dependent variables	
	X1 Smix (%)	X2 Sonication time (S)
1	45	20
2	50	28.7868
3	45	50
4	45	50
5	40	28.7868
6	40	71.2132
7	52.0711	50
8	45	50
9	45	50
10	50	71.2132
11	45	80
12	37.9289	50
13	45	50

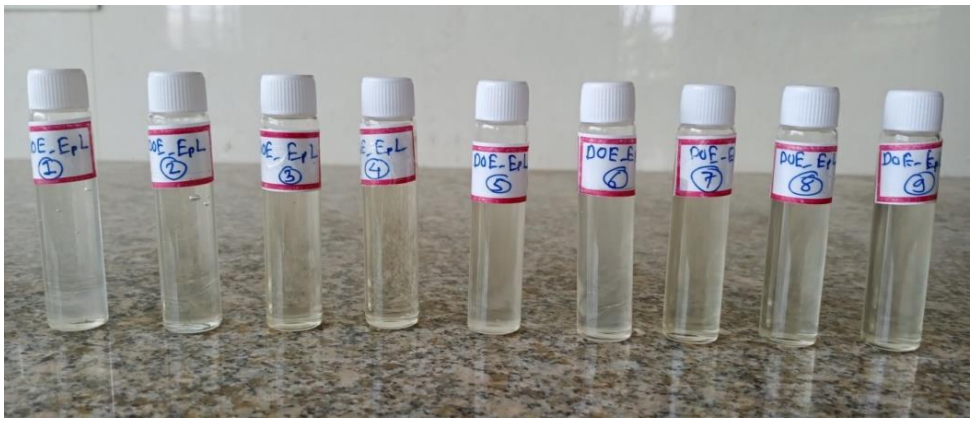


Fig. 3: Optimized eplerenone nanoemulsions

Thermodynamic stability studies

To evaluate the formulations' physical stability, several thermodynamic stability tests were conducted [30].

Cycle of heating-cooling

To evaluate the stability of the formulations, they are kept at 4 °C in the refrigerator and at 45 °C, each maintained for at least 48 h. Six cycles of alternating temperatures were followed [30].

Test for centrifugation

Phase separation and drug settling were investigated by centrifuging nanoemulsions at 3000 rpm for 30 min [30].

Freeze-thaw cycle

Three freeze-thaw cycles, with temperatures ranging from-21 °C to 25 °C, were used for storage over 48 h [30].

Characterization of eplerenone nanoemulsion

Eplerenone nanoemulsion droplet size measurement

Malvern particle size distribution apparatus (Zetasizer ver. 6.20, Model: MAL1051945) was utilised for the droplet size of the nanoemulsions, with each size value reported as the mean±SD of three samples. The Polydispersity Index was computed to assess the uniformity of particle diameters [30, 31].

ζ Potential measurement of eplerenone nanoemulsion droplets

The ζ potential of eplerenone nanoemulsion was measured using electrophoretic light scattering with a Malvern Zetasizer (Malvern Instruments, Ltd., UK). A dynamic light scattering particle size analyser set to 633 nm was employed, maintaining a consistent electrical field of 1 volt throughout the experiment. The nanoemulsion was diluted at a 1:100 ratio using pre-filtered, double-distilled water. Data were reported as mean±SD from three independent measurements [30, 31].

Determination of viscosity

At 25 °C, the viscosity of the eplerenone Nanoemulsions was measured using a Brookfield RST rheometer (Brookfield Engineering Laboratories, Mumbai) that had a C50-1 spindle attached to it. Measurements were taken in triplicate [30].

Refractive index

The system's refractive index was ascertained by applying one drop of the nanoemulsion in triplicate to the slide at a temperature of 25 °C using a refractometer (Cyber-Lab, Cyber AB, Hyderabad) [31].

pH measurement

The apparent pH of the eplerenone nanoemulsions was measured in triplicate at 25 °C using a pH meter (Systronics, model 802, India) [30].

Transmission electron microscopy (TEM)

eplerenone Nanoemulsion's morphology and structure were investigated. A point-to-point-separable Gatan 626 cryo specimen holder electron microscope (TECNAI 12, Fei Company, The Netherlands, Software: Tecnai Imaging and Analysis, Source: Tungsten Filament) running at 20–120 kV is used to determine the dimensions and form of the eplerenone Nanoemulsion, diffraction modes and bright-field imaging methods were used [31].

RESULTS AND DISCUSSION

Screening of eplerenone nanoemulsion components

Each component of the nanoemulsion was carefully chosen by the guidelines provided by the US Food and Drug Administration (FDA) for compliance and pharmaceutical acceptability of nanoemulsion components for topical administration. By going through this selection process, the nanoemulsion formulation is guaranteed to meet the exacting safety, efficacy, and quality standards needed to receive regulatory approval. Following FDA guidelines guarantees that the formulation is compatible with skin physiology, minimizes potential adverse effects, and improves topical delivery of the drug.

Screening of oils

When choosing oils for nanoemulsion formulations, it is vital to consider the drug's ability to dissolve in the oil phase. This factor is critical because the solubility of the oil phase directly influences the nanoemulsion's ability to maintain the drug in a soluble state throughout its shelf-life and during application. Generally, hydrophilic drugs work better in water-in-oil nanoemulsions because they dissolve easily in aqueous solutions but poorly in oils. Conversely, lipophilic drugs that dissolve well in oils but poorly in water are best formulated in oil-in-water nanoemulsions. A drug's solubility in different formulation components must be carefully assessed before a drug is incorporated into a nanoemulsion system, which is an important step in the process. Minimizing formulation volume is important because it allows for effective drug delivery through nanoemulsion. Upon diluting the nanoemulsion, the surfactant's or co-surfactant's solvent capacity may decrease, which could lead to precipitation if these components are involved in solubilizing the drug. It is crucial to take into account factors that affect drug incorporation capability, maintain the system's capacity for aqueous monophasic dilution, and reduce the risk of drug's settling or crystallization in dilute systems to develop stable and suitably low-volume nanoemulsions for drug delivery applications. In other words, the drug's solubility in the oil phase served as the basis for selecting the oils [32].

It was found that eplerenone was soluble in several oils (table 6). Semi-synthetic oils were chosen for the current study to determine which oil had the best drug Solubility. In comparison to the other oils, Kollicream®OA (171.37±0.92 mg/ml) and Paceol (169.32+2.22 mg/ml) were found to have the highest levels of eplerenone Solubility. It was shown that, in contrast to natural oils, the semi-synthetically produced oils had a higher solubility of eplerenone [33, 34]. It was demonstrated that eplerenone was much less soluble in propanediol, isopropyl myristate, coconut oil, sesame oil, castor oil, and ethanediol. With time, normal intermediate-chain triglyceride oils are being successfully replaced by new semi-synthetic intermediate-chain compounds with surfactant potentials [35]. Consequently, Kollicream®OA was chosen as the oil phase for synthesizing the eplerenone Nanoemulsion because of its high emulsification potential and ability to solubilize eplerenone. Paceol was rejected due to its low emulsifying capabilities with surfactants and co-surfactants.

Table 6: Eplerenone's solubility in different constituents

Components	Solubility in mg/ml[a]
Oils	
Kollicream®OA	171.37 ±0.92
Paceol	169.32±2.22
Iso Propyl Myristate	15.81±0.65
Castor Oil	2.72±0.15
Coconut Oil	1.15 ±0.14
Sesame Oil	1.62 ±0.14
Ethanediol	1.46±0.11
Olive Oil	1.28±0.034
Propanediol	1.36±0.090
Surfactants	
Tween 20	126.51± 2.24
Kolliphor®EL	36.26 ±1.03
Kolliphor®RH 40	4.53± 0.11
PEG 200	3.42± 0.20
Labrafil M1944	3.35± 0.47
Kollisolv®PEG400	3.21±0.23
Propylene Glycol	1.34±0.19
Glycerine	1.51±0.09
Lauroglycol™ FCC	1.78± 0.12
PEG 400	3.03±0.19
Co-Surfactants	
Transcutol®P	180.8±1.11
Isopropyl Alcohol	14±0.28
Ethanol	2.52±0.14

[A data are expressed as mg/ml±SD Mean±SD, n = 3]

Screening of surfactants

Glycerine, propylene glycol, Kollisolv®PEG400, Lauroglycol™ FCC, Tween20, Kolliphor®EL, Kolliphor®RH40, PEG 200, Labrafil M1944, and Kollisolv®PEG400 are the ten non-ionic surfactants whose solubilisation capacities were assessed in the current study. Non-ionic surfactants are less irritant and damaging than their anionic and especially cationic analogues, often used in place of anionic surfactants [36]. The surfactant-surfactant interaction may diminish as concentrations increase. Non-ionic surfactants were chosen as they restrict changes in pH and ionic strength, as well as their reputation for being safe and compatible with biological systems. Due to toxicological reasons, ionic surfactants were not used in this experiment. Conversely, some writers have chosen surfactants according to how well they bind drugs [37]. We claim that another critical factor is the oil's solubility in an emulsifier. Emulsifiers must have a strong affinity for the oil phase and effective drug solubilisation. The surfactant chosen should have a large nanoemulsion area when used independently to dissolve the oil phase without requiring a co-emulsifier. The emulsifier's capability to form nanoemulsions increases as the nanoemulsion area in the phase diagram expands.

The surfactants Kolliphor®EL and Tween 20 were chosen for the nanoemulsion development process because they were the best at solubilising Kollicream®OA, while Paceol remained insoluble. Surfactant-oil miscibility can therefore be used in this situation as a preliminary indicator of nanoemulsion production capacity. To synthesise the o/w nanoemulsions, the HLB value is desirable to be greater than 10. This significantly affected the choice of surfactant. When choosing emulsifiers, several factors are considered, such as their capacity to dissolve in both oil and water, HLB value, and their lower toxicity compared to other options; when preparing an o/w nanoemulsion, it is important to follow the proper steps. [36]. Non-ionic surfactants with HLB values ranging from 8 to 16 are recommended. In this investigation, we used Kolliphor®EL (HLB between 12 and 14) and Tween 20 (HLB 16) as the surfactants.

Table 7: Emulsification efficacy of surfactant with selected oil (Oil 1: surfactant 3) w/w

Surfactant (3% w/w)	Oily phases (1% w/w)					
	Kollicream®OA			Paceol		
	Dispersibility and presence	Self-emulsification period (min)	Score	Dispersibility and presence	Self-emulsification period (min)	Score
Kolliphor®EL	Bluish emulsion formed	1-2 min	+++Very good	Turbid	more than 2 min	-Poor
Tween 20	Turbid emulsion	3-4 min	+Good	Turbid	more than 2 min	-Poor

Table 8: Emulsification efficiencies of surfactants–co-surfactant combinations with oil

Co-surfactant (1% w/w)	Oily phases (1% w/w) Kollicream®OA					
	Surfactant (2% w/w)					
	Kolliphor®EL			Tween 20		
	Dispersibility and presence	Self-emulsification period (min)	Score	Dispersibility and presence	Self-emulsification period (min)	Score
Transcutol®P	Clear nanoemulsion formed	<1 min	++++ Very good	Turbid	more than 2 min	-Poor

(Oil 1: Surfactant 2: Co-surfactant 1) w/w

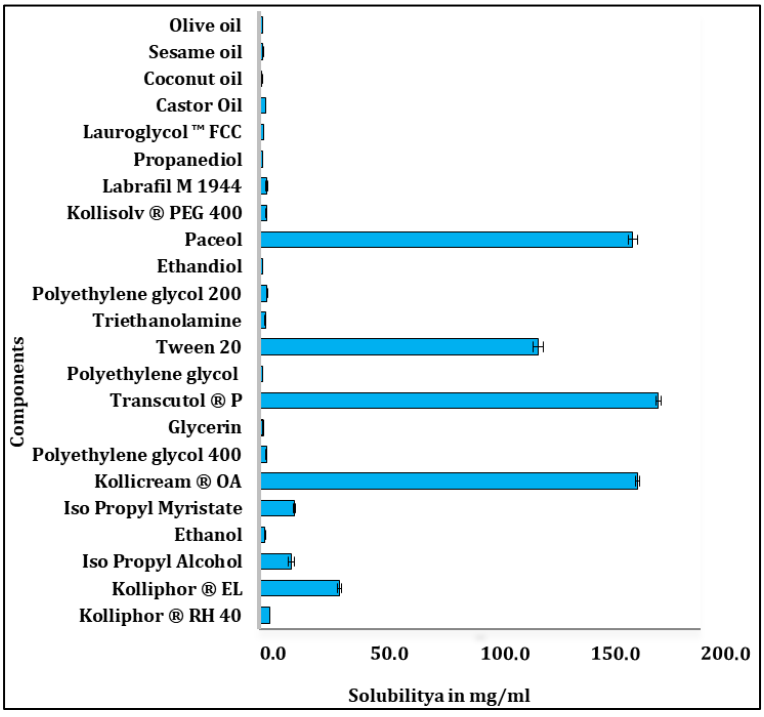


Fig. 4: Solubility of eplerenone in several oils, surfactants, and co-surfactants, [[a]Data are expressed as mean±SD, n = 3]

Screening of co-surfactants

Co-surfactants are essential since single surfactants regularly struggle to maintain a stable fluid layer and achieve a quick drop in interfacial tension. Co-surfactants are important because they reduce interfacial tension and give the interfacial layer the elasticity it needs to accept the different curvatures that are necessary for the generation of nanoemulsions. The unique characteristics of Transcutol®P, with its HLB value of 4.2, make it a valuable asset in our investigation, enhancing the stability of nanoemulsions and offering formulation flexibility [38].

Co-surfactants are incorporated to enhance the flexibility of the interfacial film, a task that surfactants alone cannot accomplish. They also aid in oil solubilisation by altering the oil-water interface's curve. Co-surfactants' ability to modify the way lipophilic drugs or therapeutic agents separate into the aqueous and oil phases makes their selection extremely important [39]. Single-chain surfactants frequently cannot effectively lower the o/w interfacial tension, preventing nanoemulsion formation [40]. Surfactants can be used to lower interfacial tension and increase the flexibility at the interface. When the concentration of surfactants is low, incorporating co-surfactants offers a unique method to create nanoemulsion systems [1, 2, 6, 9, 41].

While co-surfactants are necessary, some studies suggest that a single surfactant may be preferable over a mixture of surfactants in this study. A single surfactant exhibits greater stability in the nanoemulsion formulation than a mixture of surfactants [42]. Nanoemulsion systems are made with them at low surfactant concentrations [38]. As co-surfactants, short-to intermediate-chain alcohols are widely used to decrease interfacial tension and increase contract fluidity [1, 2, 35, 41]. More oil permeates in this region, driven by the hydrocarbon ends. These molecules can interact and alter the packing of surfactant monolayers at the interface, thereby modifying interfacial energy and curvature due to their terminal hydroxyl group, short hydrophobic chain, and amphiphilic behavior. Alcohols, capable of moving between the oil and aqueous phases, enhance miscibility. Therefore, Transcutol®P was chosen as a co-surfactant [43].

Effect of the Smix mass ratio in nanoemulsion region of a pseudo-ternary phase diagram

One method to demonstrate the formation of a nanoemulsion is using a pseudo-ternary phase diagram. In our study, Kollicream®OA served as the oil phase, while Kolliphor®EL and Transcutol®P functioned as the surfactant and co-surfactant, respectively. Analysis of the phase diagrams indicated the presence of a nanoemulsion area. Several researchers have previously documented that using surfactants alone often leads to a limited nanoemulsion area. Hence, this study took careful measures to avoid achieving a Smix ratio of 1:0 without including a co-surfactant. The primary objective of this research was to investigate nanoemulsion formation, specifically concerning incorporating a co-surfactant. Phase diagram analysis revealed that, as shown in fig. 1A and 1C, the largest region for nanoemulsions was identified in Smix proportions of 1:1 and 1:3. The maximum amounts of oil that could be soluble can be seen in these diagrams. Particularly, fig. 1A displays a 26% wt/wt solubilisation capacity at a 40% w/w Smix ratio for 1:1, and fig. 1C reveals a 22% w/w solubility at a 51% w/w Smix ratio for 1:3. Greater emulsification efficiency inside the system is shown by an increased nanoemulsion area. The results in Tables 6 and 8 show that Transcutol®P performed better than the other groups, probably because of its high eplerenone solubility. As a significant result of their reduced solubility, other co-surfactants, in contrast, showed reduced efficiency. Increasing the surfactant concentration at a 3:1 Smix ratio (fig. 1D) resulted in a smaller nanoemulsion area than the 1:1 Smix ratio. However, with a surfactant concentration of 40% w/w of Smix, this ratio's maximum amount of oil dissolved was 26% w/w. When the surfactant concentration was increased relative to the co-surfactant, a decrease in the nanoemulsion region was observed in the 3:1 blend ratio, while a slight increase was detected in the 3:2 blend ratio. This suggests that increasing the surfactant concentration did not effectively increase the nanoemulsion area, indicating that optimal emulsification was not achieved. In contrast, the nanoemulsion area expanded when maintaining the 1:1 mixture ratio.

Therefore, experimenting with a 4:1 Smix ratio was unnecessary. Consequently, the boundaries of single-phase nanoemulsion zones are dictated by the composition of a singular surfactant [42]. A decrease in the nanoemulsion region was noted after the surfactant concentration of Smix was raised from 1:1 to 3:1. One possible explanation is a low concentration of co-surfactant, which would reduce interfacial tension and give the interface and nanoemulsion region more flexibility [43, 44]. When analysing the nanoemulsion region about the entire area, it was observed that the reduction in the area was seen as the concentration of co-surfactant increased from Smix 1:1 to Smix 1:2. The area expanded when the co-surfactant concentration was raised even more, resulting in a Smix ratio of 1:3. Smix 1:2 required 42% w/w for 5 % w/w oil solubilisation, whereas Smix 1:3 only needed 40 percent w/w. The higher concentration of Transcutol®P in the 1:2 mixture and the increased surfactant concentration in the 3:1 mixture are likely contributors to the smaller nanoemulsion area. Phase diagrams showing an extension of the nanoemulsion area towards an aqueous-rich region led to more diluted formulations. The mass ratio of the co-surfactant and surfactant significantly impacted the phase characteristics [30]. The nature and amounts of oil used are other aspects [45]. The maximum nanoemulsion area was observed with Smix ratios of 1:1 and 1:3, in contrast to other ratios. This outcome is attributed to surfactant and co-surfactant packing variations at the oil-in-water (o/w) interface. Therefore, selecting an appropriate surfactant concentration is crucial for achieving maximum transdermal flux of lipophilic drugs. This situation is typically avoided in formulations containing the maximum surfactant because it reduces the drug's affinity for the vehicle and intensifies its thermophysics action. Therefore, careful optimisation of nanoemulsions is essential. Phase diagrams visually illustrate how Smix decreases interfacial tension and increases interfacial area and dispersion entropy. Introducing Smix can significantly lower the free energy of the nanoemulsion system to a minimum concentration, ensuring thermodynamic stability and presenting a promising approach for efficient drug delivery [46].

Table 9: **The properties and assessment of the formulations for eplerenone nanoemulsions**

Formulation code	Mean globule size (nm)[a]	Polydispersity index[a]	Viscosity[a] (Pa*s)	pH[a]	Per cent drug content[a]
E1	275 ± 3.45	0.345 ± 0.09	6.31 ± 0.22	6.80 ± 0.03	98.23 ±0.34
E2	315 ±5.52	0.368±0.01	6.34 ± 0.38	5.84 ± 0.07	92.5 ±0.45
E3	342 ±4.54	0.386±0.01	6.51 ± 0.31	5.56 ± 0.01	96.68 ±0.87
E4	124 ±4.74	0.128±0.05	6.61 ±0.27	6.12 ± 0.07	98.45 ±0.78
E5	109 ±3.21	0.107±0.04	6.78 ± 0.24	6.32 ± 0.08	99.48±0.68
F1	330±4.29	0.387±0.07	7.02 ± 0.31	5.98± 0.09	101.5±1.25
F2	150 ± 2.44	0.138±0.02	7.25 ± 0.22	6.95 ± 0.04	106±1.45
F3	328 ±3.51	0.285±0.05	7.65 ±0.22	6.89 ± 0.06	97.87±0.87
F4	112±2.36	0.112±0.03	7.78 ± 0.20	6.48 ± 0.08	94.48±0.58

[[a]Data are expressed as Mean±SD, n = 3]

Table 10: **The properties and assessment of the optimized formulations for eplerenone nanoemulsions**

Formulation code	Mean globule size (nm)[a]	Polydispersity index[a]	Viscosity[a] (Pa*s)	pH[a]	Per cent drug content[a]
DOE_eplerenone_1	347.6±2.16	0.424 ±0.0040	6.57±0.17	6.88 ±0.081	98.4±0.44
DOE_eplerenone_2	113.1 ±2.53	0.234 ±0.0035	7.46 ±0.18	6.53±0.041	102.4± 0.61
DOE_eplerenone_3	122.4 ±2.65	0.226 ±0.0055	6.96 ±0.16	6.79 ±0.008	100.9± 0.35
DOE_eplerenone_4	133.6 ±0.57	0.237 ±0.0036	7.24 ±0.31	5.88 ±0.09	100.1± 0.95
DOE_eplerenone_5	148.6 ±1.58	0.322 ±0.0015	7.86 ±0.22	6.35 ±0.10	99.5± 0.70
DOE_eplerenone_6	121.3 ±1.17	0.150 ±0.0020	6.54 ±0.23	5.45 ±0.24	98.9± 0.20
DOE_eplerenone_7	124.3 ±0.59	0.243 ±0.0021	5.81 ±0.85	6.50 ±0.22	99.3± 0.38
DOE_eplerenone_8	109.2 ±0.85	0.134 ±0.0031	7.57 ±0.15	6.68 ±0.17	100.4± 0.71
DOE_eplerenone_9	109.1 ±0.21	0.129 ±0.0010	7.23 ±0.25	6.64 ±0.22	99.3± 0.46

[[a]Data are expressed as Mean±SD, n = 3]

Thermodynamic stability tests

Stress testing is necessary to ensure the formulations are stable and do not cause any risks. A few selective nanoemulsions were carefully chosen from the phase diagram's o/w nanoemulsion area at Smix 1:1 and 1:3 as they demonstrated the maximum nanoemulsion area. Then, these nanoemulsions underwent a series of thermodynamic stability assessments, such as freeze-thaw cycles, the heating-cooling cycle, and centrifugation. Stress testing is necessary to ensure the formulations are stable and do not cause any risks. Phase separation, turbidity, creaming, or cracking have all been noted in specific formulations. E4 has been chosen for additional optimisation and determined to be stable (data hidden). Due to thermodynamic stability, the nanoemulsion has a longer shelf life than conventional emulsions. It sets them apart from emulsions with kinetic stability that are going to phase separately in the future [31, 32]. Table 3 lists the ingredients in these formulations, and optimised nanoemulsions are shown in fig. 3. They have undergone thermodynamic stability tests and passed stress testing.

Eplerenone nanoemulsions and optimized nanoemulsion characterization

eplerenone nanoemulsion droplet size measurement

At a 90° angle and 25 °C temperature, light scattering was observed with the laser light scattering phenomenon, which examines changes in light scattering. Analysing droplet size was performed using diluted nanoemulsion samples, each 100 times diluted. Information about the polydispersity index and average droplet size was collected. The polydispersity index is used to determine the size distribution of droplets, and the size of the droplets is measured in nanometers. Larger droplet sizes are linked to more significant variability in the distribution of droplet sizes [31, 33].

ζ Potential measurement of eplerenone nanoemulsion droplets

The ζ potential of eplerenone nanoemulsions was assessed using a Zeta sizer. The nanoemulsions were diluted 1:100 v/v in distilled water and vortexed before measurement. Three independent analyses were conducted. The absolute value indicates the magnitude of the surface charge. Higher absolute ζ potential values, regardless of their polarity, typically specify improved stability as particles with similar charges repel each other, thereby inhibiting flocculation or aggregation [31].

Determination of viscosity

As the oil content increased from 5%w/w to 20%w/w, a rise in viscosity was observed in the nanoemulsions (table 6). Notably, formulation Optimized_eplerenone_7 exhibited significantly lower viscosity than Optimized_eplerenone_4 and Optimized_eplerenone_9 ($p<0.05$), which may be attributed to its lower Smix content. Overall, the formulations showed optimal viscosity profiles.

Refractive index

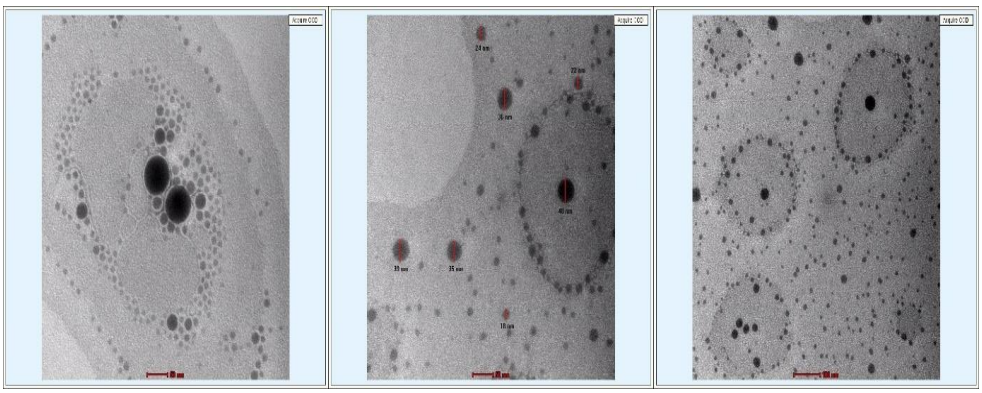
The refractive index is a metric for system homogeneity that represents the collective properties of the nanoemulsion's constituent parts. All of the improved formulations had the same average refractive index values. However, the refractive index of the Optimized eplerenone 4, 6, and 9–13 formulations improved slightly (table 3). A decrease in water content, which usually yields a lower refractive index, may cause this alteration.

pH measurement

The apparent pH of eplerenone Nanoemulsions and optimised eplerenone Nanoemulsions was determined in triplicate. Every formulation has a pH of ≥5.56±0.01 and ≤6.88±0.081 (Tables 9 and 10).

Transmission electron microscopy (TEM)

Optimised eplerenone 4 was dropped onto a carbon-covered grid for TEM analysis. Following the appropriate dilution with water, the sample was exposed to the reagent and left to stand for 30 S. After the coating was applied [31].



A **B** **C**

Fig. 5: Transmission electron microscopy of optimized DOE_EpL 4 (A, B) Scale bar is 50 nm; (C) Scale bar is 100 nm

CONCLUSION

An effective nanoemulsion formulation depends on careful component selection. The main conclusion drawn from this study showed the application of visual appraisal and grading systems to assess the final appearance, dispersibility, and ease of emulsification for eradicating the toxicity and irritation that nanoemulsions may cause. Kolliphor®EL (3% w/w) was able to emulsify Kollicream®OA (1% w/w), and Paceol failed. As well, Smix [Kolliphor®EL (2% w/w) and Transcutol®P (1% w/w)] were able to emulsify Kollicream®OA (1% w/w). For the synthesis of the ideal nanoemulsion of eplerenone, selected excipients were from Kollicream®OA, Kolliphor®EL, and Transcutol®P. Selected, optimised formulations were characterised by zeta potential, droplet size, morphology, viscosity, pH, and polydispersity index. The study offered convincing proof of how correct ratios of oil, surfactants, and co-surfactants can be used to achieve the required results, and Optimized nanoemulsions will be used in the formulation of eplerenone's Nanoemulsion Gel for transdermal application.

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AUTHORS CONTRIBUTIONS

Mahesh T. Gaikwad is the primary researcher who carried out the entire research work throughout the project and wrote the manuscript under the guidance of Rajendra P. Marathe. Inayat B. Pathan contributed in data analysis.

CONFLICT OF INTERESTS

Declared none

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