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Development and Optimisation of Nanoemulsion as carrier for Cucurbitacin

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ABSTRACT:

The purpose associated with this study was to create an optimised nanoemulsion formulation with optimised components wt. % of oil: surfactant-cosurfactant: water, which would be stable, efficient, efficacious and more suitable for delivering the lipophilic Antidiabetic Bioactive Compounds (ABC) via topical application. The optimization was done by using studies over parameters like solubility, stability and emulsification power. Pseudoternary phase diagram was developed for perfect and honed selection of components and further the ratios of selected components was precisely optimised. Organic phase of nanoemulsion was selected as sefsol218 with its maximum solubilising power of drug. Tween80 was selected as surfactant for sefsol218 on basis of area of emulsion on phase diagram, solubility of sefsol218 in surfactant and solubilising power of surfactant to drug CBT. Cosurfactant screened was Transcutol. It was based on nanoemulsion available area (NEAR) on phase diagram with 1:1 Smix ratio of tween80 and six different cosurfactants mixed with sefsol218 and titrated with water. Different components wt. rations were optimized by titrating mixture of sefsol218 and various Smix of tween80 and Transcutol and plotted on Pseudoternary phase diagram to find out the area of nanoemulsion region. 29 formulation were selected and tested for their metastability by thermodynamic cycle testing. Total 11 formulation passed the test and selected as placebo formulations for the study.

I. INTRODUCTION

Isotropic systems of two immiscible liquids as oil & aqueous phases with nano scaled droplets (10-100 nm) are nanoemulsions (NEs) (Chime, Kenechukwu, and Attama 2014). These are made thermodynamically stable by using optimised surfactant and co-surfactant blend rations (Smix) which reduces interfacial tensions efficiently(Ashaolu 2021). The optimised stable NEs are transparent (or translucent)(Prasad, Mohanta, and Sudhakar 2019). NEs have high solubilising power of drug side by side longer stability, spontaneous preparation that is why these crafted wide applications for drug delivery. For solubility and bioavailability of the lipophilic drug these are treated as most efficient tools either by oral or by transdermal delivery(Reddy and Tripura Sundari 2019). They are being investigated for applications through body cavities like ocular, pulmonary, nasal & vaginal. Their parenteral preparations are also a mean of efficient drug delivery(Kendre and Satav 2019).

The reviews reveal that credentials of NEs are attributed to screening of oil phase, surfactants and co-

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surfactants(Azeem, Rizwan, et al. 2009). How screening of excipients contribute for NE formulation development, was the main objective of this study. Cucurbitacin (CBT) was selected as a model lipophilic drug. In classical system these drug requires high surfactant concentration, which is toxicity and irritant for dermal applications. Determination of optimum excipients in mass ratio would lead to develop better formulation with desirable attributes (Gopinath and Naidu 2011).

II. MATERIALS AND METHODS

2.1. Components:

CBT was bought from Sigma-Aldrich Chemicals, Bangalore. Sefsol-218®, Stepan D-50, Tricetin, Peceol®, Miglyol 812® Labrafac® were gift samples. Cremophore, Tween 80, Tween 20, Tween 40, Labrasol, Transcutol, Plurol oleique was procured from R V Northland Institute, Grt. NOIDA. All other chemicals and reagents used were of analytical grade obtained from Merck, Mumbai. Water was obtained from Milli-Q water purification system (Millipore, MA).

2.2. Screening of Oil:

2ml of each solvents are taken 5ml stoppered viol separately. Added drug in excess amount in each viol. For mixing, vortex mixture is used. After proper mixing the viols with mixture are kept on isothermal shaker at 25.0±1.0 ^{0}C for 75 h for equilibrium. These samples centrifuged after removing from isothermal shaker at 3000 rpm for 15 minutes. The supernatant was taken and filtered through 0.22 µm membrane filter. The concentration of CBT drug was determined by using HPLC (Sanjar Alam et al. 2012).

2.3. Screening of Surfactants:

As per GRAS for skin 5 surfactants were selected for screening.

Power of emulsification- Five surfactants were careened and tested for nanoemulsion formulation: Labrasol, Cremophor EL, Tween 20, 60, and 80. One of these was selected by taking in 2.5 ml of water and adding each of these as 15% w/w and 4 µl of selected oil with vigorous vortexing. If a one-phase transparent mix was got, start to add more oil until hazy mixture found (Fernandes et al. 2021).

Power of drug solubilisation- Next step drug solubility was assessed in each of five surfactants by mixing excess amount of drug in 2 ml of surfactant in 5ml stoppered vials. Procedure of testing was followed as in section 3.2 and the concentration of CBT drug were determined by using HPLC (Bali, Ali, and Ali 2010).

The selected surfactant was again assessed by taking in 1:1 ratio with organic phase, vortexed for 5 minutes, and kept for 24 hours at 25^{0}C temperature as part of a miscibility study. The product was analysed for phase separation and color change after 24 hours (Azeem, Ahmad, et al. 2009).

2.4. Screening of Cosurfactants:

The selected surfactant tween 80 (HLB-15) was tested with 6 different co surfactants (Transcutol-4.20, Plurol-oleique-3.0, and Propylene Glycol-2.5) as:

RHLB: in range of 9 to 12 is reported for o/w nanoemulsions (Kabri et al. 2011).

Power to reduce concentration of surfactant, done by using a 1:1 Smix ratio of surfactant and cosurfactant, Pseudoternary phase diagrams were created(Xi et al., n.d.).

2.5. Optimisation of Smix Ratio of Surfactant and Cosurfactant:

Effect of Surfactant and Cosurfactant Mass Ratio on Nanoemulsion Formation was assessed by taking the weight ratios of surfactant and cosurfactant 3:1, 2:1, 1:1, 1:0, 1:2, and 1:3. For comprehensive phase diagram analysis, these Smix ratios were chosen in decreasing surfactant concentration relative to cosurfactant and increasing cosurfactant concentration relative to surfactant (Mostafa et al. 2015).

2.6. Optimisation of selected components of nanoemulsion:

total of 12 combinations of oil and Smix weight ratios (1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 6:4 (1:0.7), 7:3 (1:0.43), and 9:1 were used to clearly define phase boundaries in phase diagrams. The pseudoternary phase diagrams were created using data of aqueous titration, which includes adding water to each weight ratio of oil and Smix(Choudhury et al. 2014).

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In order to plot each phase diagram, sixteen distinct ratios of oil and Smix were first vortexed to make a transparent and homogenous solution. This was done after the slow aqueous phase titration was performed using a micropipette while continuous stirring was carried out.

The mixed ratios of oil to Smix was progressively titrated with water. By visual observations the decisions taken based on the following criteria:

- The o/w Nanoemulsions (NE) marked ok, as long as the nanoemulsion can be observed transparent and flowing readily. The nanoemulsion must be visibly transparent.
- If any emulsion has the appearance of being milky or foggy, or if there is any trace of phase separation visible, it will be designated as not acceptable. Marked Not OK.

2.7. Screening of stable formulations:

Marked formulations were subjected to thermodynamic stability testings' to screen out most stable formulations physically and thermodynamically (Gurpreet and Singh 2018).

Centrifugation study:

After centrifuging the formulations at 3,500 rpm to 5000 rpm for 30 minutes, we looked for signs of phase separation if any.

Heating cooling cycles (Six cycles)

It tests nanoemulsion stability after heating and cooling the mixtures. The temperature must be cycled 44°C and 4°C (Refrigerator Temperature). Storage must not be less than 48 hrs. at every temperature climb and fall (Rai et al. 2018) (Harwansh et al. 2015).

Freeze-thaw cycle:

This involved exposing the formulations to two temperature changes, -20 C and 25 C, for not less than 48 hours at each temperature. Each set of mixture must pass three rounds to determine its stability. (Harwansh et al. 2015).

2.8. Development of drug loaded nanoemulsion formulation:

The ratios of components of placebo nanoemulsions were taken as stable optimised formulations. These rations are redeveloped by taking stock solution of drug in sefsol218.The components, Drug CBT solubilized Sefsol218, Smix (surfactant & cosurfactant) and water were added in the same % ratios as optimised and stable placebo formulation was recorded.

III. RESULT AND DISCUSSION

The most important criteria for selection of all the nanoemulsion components is that all the excipients should be pharmaceutically acceptable for topical application as well as oral application, etc., depending upon the requirement and falling under the generally-regarded-as-safe category.

3.1. Screening Criteria for Oil Phase:

Efforts should be made to minimise the volume of the formulation in order to effectively deliver the therapeutic dosage of the medicine in an encapsulated format. The drug solubilisation and load in each formulation is a major design parameter in the fabrication of nanoemulsion systems for poorly soluble pharmaceuticals. This factor is contingent upon the solubility of the drug in different components of the formulation. The capacity of nanoemulsion to sustain the medication in a solubilized state is significantly impacted by the drug's solubility within the oil phase. In the event if the surfactant and cosurfactant is playing a role in enhancing medication solubilisation, it could be a positive effect for skin penetration and will support the permeation.

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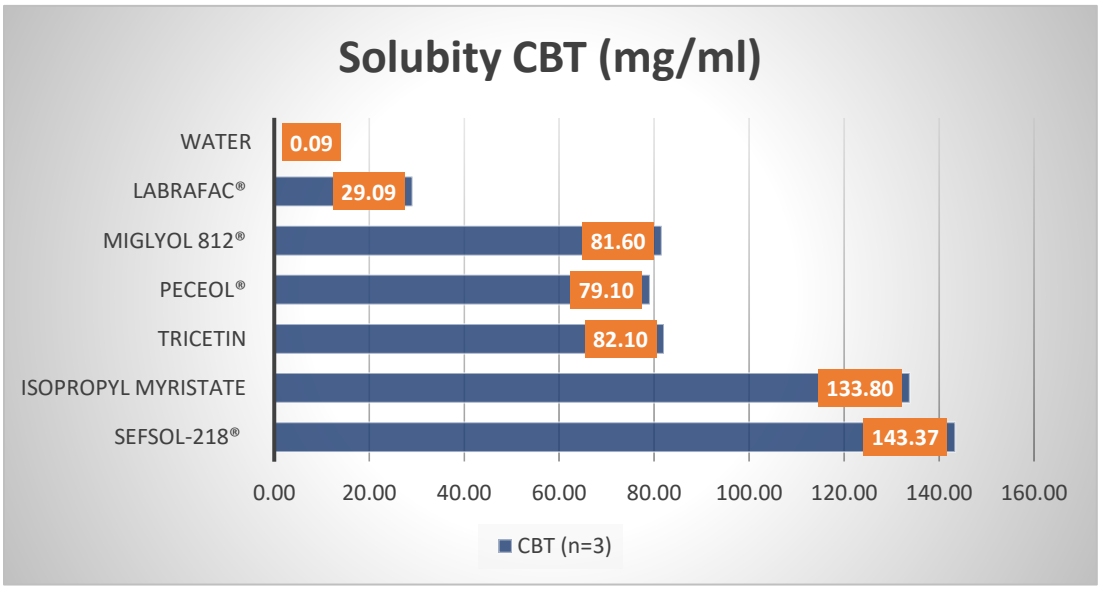

Fig.1: Drug solubilisation power of different oils

The solubility of CBT was determined in different oils (Fig.1). It was found that CBT drug is best solubilised in sefsol218. In comparison to other oils. The utility of edible oils is sometimes limited by their insufficient dissolving power for lipophilic medicines. This will demand large volume of oil side by side also demand for high concentration of surfactant and cosurfactant and it would consequence to toxicity.

3.2. Screening Criteria for Surfactants:

Proper selection of surfactant is very important because the main issue associated with nanoemulsion-based systems pertains to the toxicity of their constituent components. Excessive quantities of surfactants have the potential to induce skin irritation. This can be managed by

a. Selecting less toxic surfactant like non-ionic surfactant. Non-ionic surfactants are also knoiwn as less affected by pH change, and changes like ionic strength

b. By reducing the concentration of surfactant by taking low CMC surfactant

c. Greater than 10 HLB of surfactant stabilises the nanoemulsion (Kommuru et al. 2001). When the correct proportions of low and high HLB surfactants are used, a stable nanoemulsion can be formed in water.

After selection of oil as sefsol218, different five non-ionic surfactants named as Labrasol, Cremophor EL, Tween 20, Tween 40, and Tween 80 were chosen for screening. This is performed on three basis of three data, one is its power to develop nanoemulsion area without addition of cosurfactant (table:1.) (Fig.2), seconds is solubility of oil in surfactant w/w and third one is its power of solubilising the drug.

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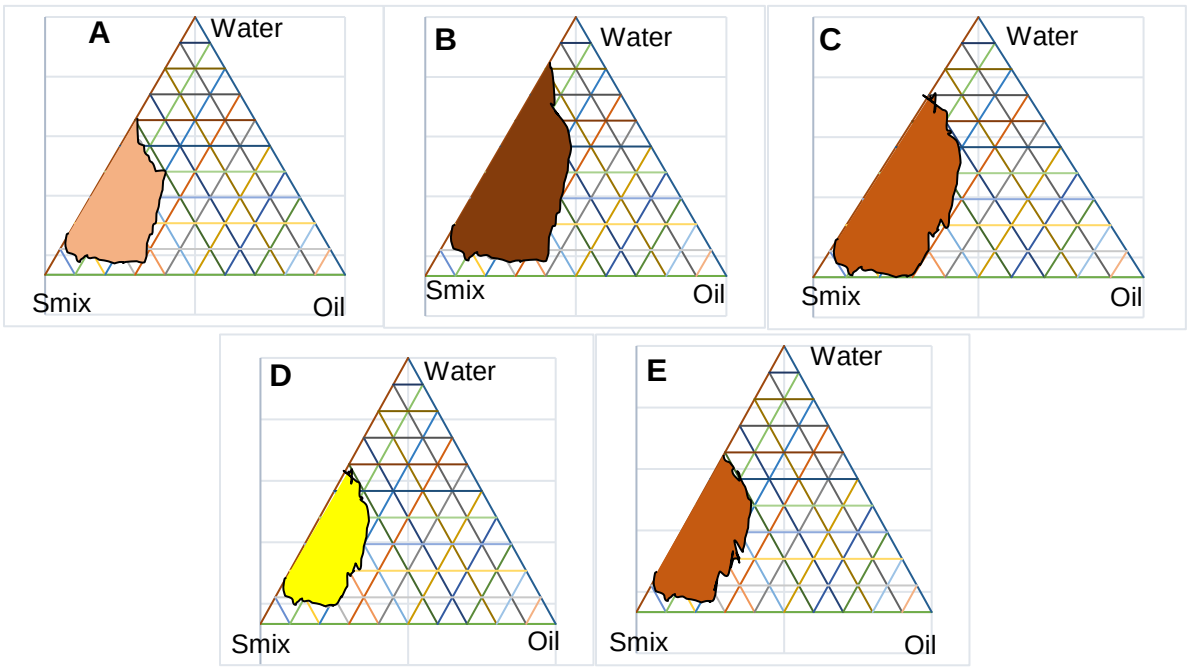

Fig.2: PTP diagrams of nanoemulsion composed of Water, Sefsol218 & different surfactants A. Labrasol; B. Tween80; C. Tween40; D. Tween20; E. Cremophore EL.

Table 1: NEAR recorded from Ternary Phase Diagram

Surfactant	LABRASOL	TWEEN 80	TWEEN 40	TWEEN 20	CHREMOPHORE EL
rea(Sq. cm)	21.16±0.52	23.58±1.39	19.05±1.25	17.82±1.11	19.80±0.66

Table 2: Solubility recorded Wt. % of sefsol218 in different surfactants

Surfactant	LABRASOL	TWEEN 80	TWEEN 40	TWEEN 20	CHREMOPHORE EL
Sefsol218 Solubility (w/w)	1.06±0.31	1.85±0.28	0.80±0.21	0.78±0.11	0.91±0.66

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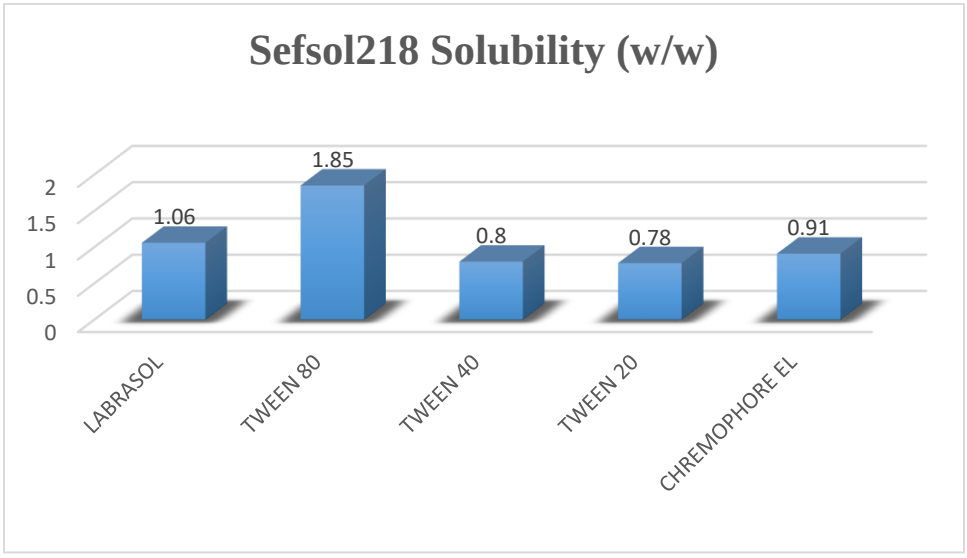

Fig.3: Solubility of oil in different surfactants A. Labrasol; B. Tween80; C. Tween40; D. Tween20; E. Cremophore EL.

Tween 80 was found the selected one surfactant for CBT drug's placebo formulation because the NEAR (Nano Emulsion Available Area) created by tween 80 is maximum with sefsol218 is 23.58±1.39 Sq. cm (fig.2) & additionally As per United State Pharmacopoeia (USP), sefsol218 is freely solubilised in tween80 (1.85±0.28 mg/ml) and Labrasol (1.06±0.31) and in comparison it is more solubilised in tween80 (Table: 2) (Fig.3). Tween80 has highest drug solubilising power (Fig.4), is 72.29 mg/ml, 81.64 mg/ml for CBT respectively. So on the basis of above results, tween80 has been selected as surfactant.

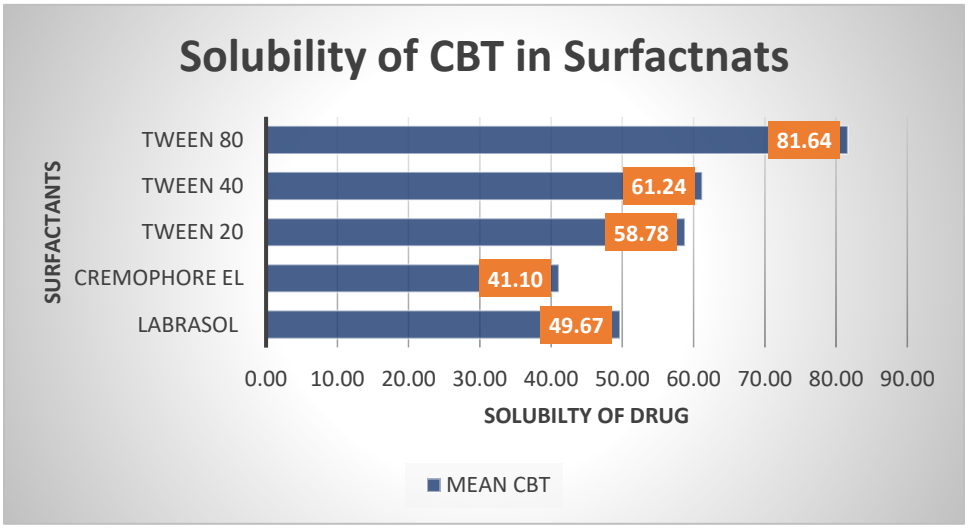

Fig.4: PTP diagrams of nanoemulsion composed of Water, Sefsol218 & different surfactants A. Labrasol; B. Tween80; C. Tween40; D. Tween20; E. Cremophore EL.

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3.3. Screening Criteria for Cosurfactants:

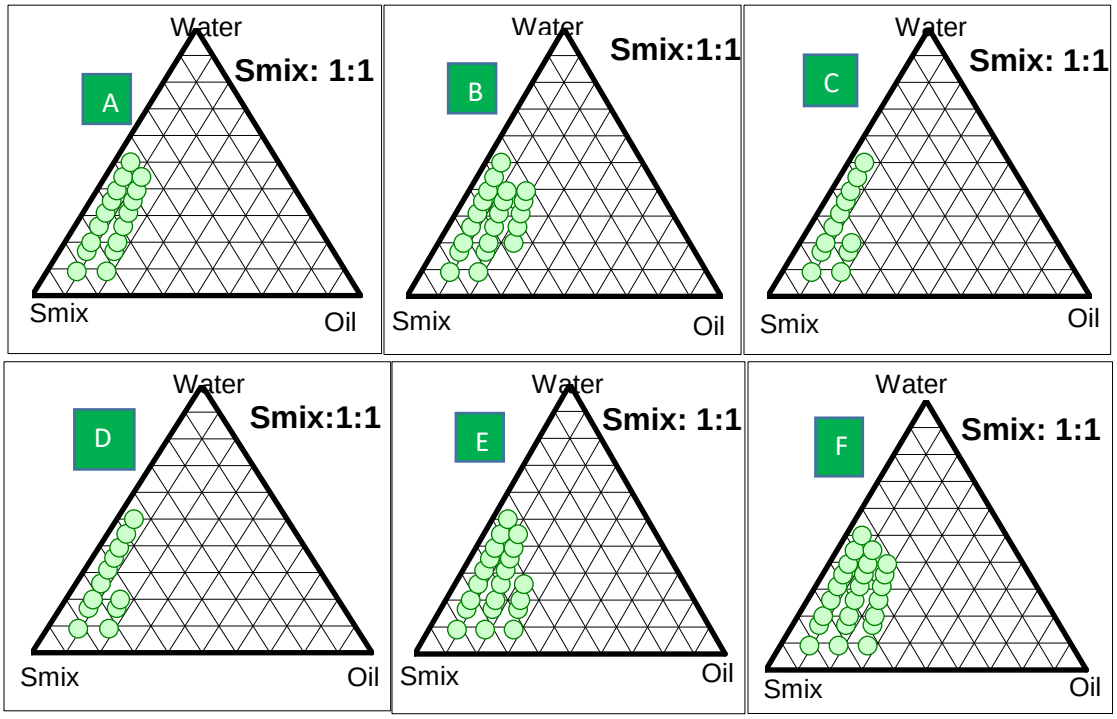

Fig. 5: PTP diagrams of nanoemulsion composed of Water, Sefsol218, Tween 80 and different Co-surfactants: A. ethanol; B. isopropyl alcohol; C. butanol; D. PEG 400; E. Plurol Oleique; F. Transcutol P, at Smix 1:1.

Individually surfactant needed for best emulsification was not sufficient and also its large concentration was intended to decrease for safety and maintaining its HLB in o/w emulsion range (8-12). The said points can be achieved by adding short carbon (3-8) chain length alcohols. These are called as co-surfactants. These has potency to reduce interfacial tension in association with surfactants. Two more thing additionally improved, fluidity and mobility of hydrocarbon chain. Due to presence of alcoholic groups, they also enhance the miscibility of water and oil phases into each other by partitioning in to both. These traits of cosurfactants are used to increase NEAR and stabilise nanoemulsions.

The screening of cosurfactants was based on the nanoemulsion available region (NEAR). At a constant Smix (1:1) with keeping the same surfactant but swapping out the cosurfactants, the size of the NEAR in the phase diagrams was compared (Fig.5). The NEAR is directly proportional to the efficiency of emulsification.

Table 3: NEAR recorded for effect of Cosurfactants at fixed 1:1 Smix with tween 80.

Surfactant	**Tween 80 (HLB-15)**					
Co-Surfactant	**Ethanol**	**Isopropyl alcohol**	**1-Butanol**	**PEG 400**	**Plurol Oleique**	**Transcutol**
Smix	1:1	1:1	1:1	1:1	1:1	1:1
Area(Sq. cm)	20.16±0.62	23.12±1.47	21.22±1.47	18±1.19	30.11±0.17	33.85±0.82

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Here we concluded that if we move from Ethanol to Isopropyl alcohol the NEAR increases due to in C-Chain, but to Butanol again it decreases. Also, increasing the number of hydroxyl groups as we move from isopropyl alcohol to PEG the NEAR decreases. Transcutol was able to give maximum NEAR as compared to other taken cosurfactants (Table: 3) (Fig. 5). Here it was confirmed that the cosurfactant can alter the NEAR and consequently the phase behaviour. On the basis of above findings, Transcutol was selected as cosurfactant.

3.4. Optimisation of NE components by Pseudoternary-phase diagrams:

Efficiency & efficacy of drug delivery and self-stability of nanoemulsions are function of components of it. So optimisation of ratio of components is required to accomplish best formulation. To determine the ideal ratio of surfactant/co-surfactant for producing quite large isotropic NE area, also known as the Nanoemulsion Available Reason (NEAR), Pseudoternary phase (PTP) diagrams were constructed utilising titrations carried out in the aqueous phase of the experiment. The amount of water that needs to be added in order to establish a water concentration that ranges up to 95% of the total volume at +5% increase. The outcomes of the visual inspections that were carried out afterwards the water-adding to the oilphase and Smix in percentage-based increments are presented in Table: 4. The following ratios of surfactant to co-surfactant were utilised in the titration: 1:0, 1:1, 1:2, 1:2, 1:3, 2:1, 3:1, and 4:1.

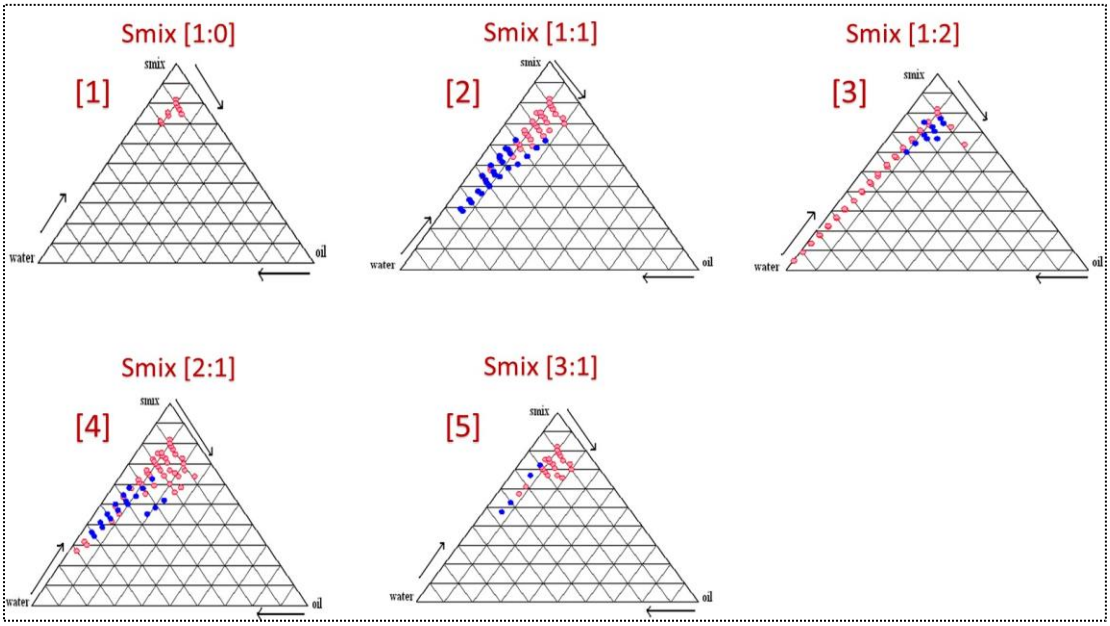

Fig. 6: *Diagrams depicting the oil-in-water nanoemulsion region at various Smix ratios*

Table: 4. NEAR recorded from Ternary Phase Diagram

Ratios	1:0	1:1	1:2	2:1	3:1
Area (Sq. cm)	**21.46±0.72**	**30.82±2.17**	**33.25±2.19**	**37.96±1.71**	**33.85±0.82**

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The use of software allowed for the construction of Pseudoternary phase diagrams (PTP) that included each Smix ratio and the water phase. (Fig.6.). NEAR is depicted in the diagram of a 1:0 Smix ratio; i.e. Tween alone is used. it is located at the apex of the PTP diagram and is very small NEAR level. The maximum amount of oil phase that could be disseminated in the preceding was only 16% w/w when utilising 67% w/w of Smix. Moving on to the next Smix ratio 1:1, i.e. cosurfactant is added now it has larger NEAR. This is due to the fact that addintion of cosurfactant results in a further lowering of the interfacial tension. In the subsequent stage of increasing the cosurfactant, that is, moving on to the Smix 2:1 stage, the NEAR increased as compared with the region in the 1:0 and 1:1 stages. Oil could be distributed at a rate of 22% by weight while utilising 52% by weight of Smix. Now continued for Smix ratio of 3:1, NEAR was little less in comparison to 2:1, but the dispersion ratio of oil with this Smix was 22% w/w with 52% w/w of Smix. This was a success. In the subsequent steps of the investigation, the Smix ratio of 4:1 NEAR was shown to have a lower NEAR value than 3:1 and 2:1, but it had a relatively high value in comparison to 1:1 and 1:0. The oil phase that can be spread at this time was 17% by weight and consisted of 67% Smix by weight.

Based on the data presented above, it was determined that the NEAR rises with increasing concentrations of surfactant as compared with co-surfactant up until a Smix ratio of 2:1, but that it starts to decrease at a Smix ratio of 3:1, and hence, further research into a Smix ratio of 4:1 was not pursued. Because of the rise in CoS, the optimal NEAR could not be determined using the Smix ratios of 1:2 and 1:3. (Akhter et al. 2008).

The conclusion that can be drawn from this is that phase diagrams express NEAR best when they are constructed on the basis of the relationship and interaction between three phases. When conditions are just right, nucleation event generation happens naturally, and the dispersions that are produced are thermodynamically stable. The ternary phase diagram demonstrates the greatest NEAR for the optimal proportion of three phases (Smix, Oil, and Water) to produce thermodynamically stable NEs, which will have a high potential for the transdermal distribution of drug.

An excessive amount of surfactants can cause sensitivity and discomfort in living skin. Therefore, the adjustment of the surfactant concentration with regard to both the reduction of surface tension and the preservation of safety. The NEs that had the optimal three phases—oil, Smix, and water—were chosen with the assistance of pseudo ternary phase diagrams. These NEs were able to hold the required quantity of CBT. (Azeem, Ahmad, et al. 2009). After this optimisation, 29 formulations (Data not shown), selected for application of next test.

3.5. Screening of stable optimised formulations

NEs were examined for thermodynamic stability (Table: 5.15.) by-heating cooling cycle (H/C), Freeze thaw cycle (F/Th) & Centrifugation (C/f) (S. Alam et al. 2010) (Mostafa et al. 2015).

In the table 5.15 the three tests are concluded with √ and X signs, first sign denotes the passed one while the second one was for failed test sign. The formulations which has passed all the three thermodynamic stability investigations has selected for further study. Here with Smix 1:0, all the formulations failed the test so these all are discarded, in the next with Smix 1:1, coded as NE3B, NE4B & NE6B has passed the three tests, so are selected. In other next with Smix 2:1, formulation coded NE1C, NE3C & NE4C NE5C, NE6C, NE8C & NE10C has passed the three tests, so are selected. In last one with Smix 3:1, formulations coded NE2D NE3D, NE5D has passed the three tests, so are selected. All these passed formulations are placebo, are reformulated with the same composition containing drug in oil phase.

3.6. Development of drug loaded nanoemulsion formulation

The oil phase stock solution in which maximum drug dissolved as per its solubility was used for drug loaded formulation development (Table: 5).

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Table 5: Optimised and thermodynamically stable NEs composition loaded with drug.

Smix		% Oil	% Smix (v/v)		% Water	
S:CoS	Codes	(v/v)	Tween 80	Transcutol	(v/v)	Drug(mg/ml)
1:1	NE3B	22.85	14.65	14.65	47.85	31.84
	NE4B	25.21	12.31	12.31	50.18	35.13
	NE6B	29.77	12.88	12.88	44.48	41.48
2:1	NE1C	14.28	26.67	13.33	28.57	19.90
	NE3C	20.51	13.19	6.59	59.71	28.58
	NE4C	23.72	14.72	7.36	54.2	33.05
	NE5C	23.07	35.89	17.95	23.07	32.15
	NE6C	25.85	14.87	7.43	51.85	36.02
	NE8C	30.3	16.66	8.33	44.71	42.22
	NE10C	30.76	17.02	8.51	43.71	42.86
3:1	NE2D	18.6	20.93	6.98	53.48	25.92
	NE3D	19.35	33.87	11.29	35.48	26.96
	NE5D	22.85	25.73	8.58	42.85	31.84

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Discloser:

The authors report no conflicts of interest or financial benefit from this work.

IV. SUMMARY AND CONCLUSION

An effective, efficient and stable nanoemulsion formulation relies on the careful selection of its constituent parts. Optimisation was focused for oil phase so that maximum amount of drug can be solubilized in minimum amount of oil in nanoemulsion. Optimisation of surfactant was mainly concern to minimize or eliminate the toxicity and irritability with efficient emulsification of oil phase. Optimisation of cosurfactant was targeted to reduce the surfactant wt. ratio and increase the action of emulsifying agent to minimized interfacial tension. Over all the study was focused to find optimised formulation of nanoemulsion components (oil: Smix: Water) by which desirable properties can be achieved for best permeation and release of drug CBT through topical drug delivery system.

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