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Design, development and evaluation of nicotinamide microemulsion based gel for topical drug delivery

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ABSTRACT

The purpose of this study was to prepare topical microemulsion based gel of Nicotinamide. Orange oil as the oil, Tween 20 as the surfactant, polyethylene glycol 200 as the cosurfactant Carbopol 934p as a gelling agent screened as phases of microemulsions, due to a good solubilizing capacity of the microemulsion systems and excellent skin permeation rate of Nicotinamide. The pseudo-ternary phase diagrams for microemulsion regions were constructed using orange oil as the oil, Tween 20 as the surfactant, polyethylene glycol 200 as the cosurfactant. Various microemulsion formulations were prepared from Surfactant and cosurfactant ratio 1:0, 1:1, 2:1, 3:1 and the abilities of various microemulsions to deliver Nicotinamide through the skin were evaluated in vitro using Franz diffusion cells fitted with Cellulose membrane. The in vitro permeation data showed that microemulsions increased the permeation rate of Nicotinamide over the control solution of Nicotinamide. The optimum formulation consisted of 1.5 % Nicotinamide, 2.85% orange oil, 17.15 % Tween 20/PEG (2:1) and 78.5 % water, showed a high permeation rate. These results indicate that the optimized formulation of Nicotinamide microemulsion may be used as a promising vehicle for topical delivery of Nicotinamide.

Key words: Microemulsion based gel, Microemulsion, Nicotinamide

INTRODUCTION

Pellagra is a nutritional deficiency disease caused by insufficient intake or absorption of nicotinamide and tryptophan, an amino acid. Its classic symptoms include dermatitis, diarrhea, dementia, and death if untreated.

Nicotinamide (NA) is also known as Vitamin B3, vitamin pp, or nicotinic acid amide. NA acts as an anti-inflammatory agent due to its anti-inflammatory action and also reduce inflammation.[2] It is used both topically and orally for the treatment of mild to moderate pain. NA also improve pigmentation, blotchiness

and redness of the aging skin. NA stabilizes epidermal barrier function and improves moisture content of the skin. [3] Moreover, NA also acts as a skin whitening agent [4] and reduce inflammation and pain. On aging skin, NA improves the surface structure of the skin

Microemulsions have been previously used in the treatment of pellagra, [8] and they are known to improve the permeation/penetration of hydrophilic and hydrophobic drugs which can be due to the availability of additional solubilization sites of the hydrophilic and lipophilic moiety of the surfactant interface film.[9] Therefore, microemulsion can be a favourable drug delivery system. Microemulsion is a single phase optically isotropic and thermodynamically stable liquid solution. These are homogeneous dispersions of water/oil type, oil/water type, and bicontinuous type. Carbopol are the most commonly used gelling agents in the pharmaceutical and cosmetic industry. To attain maximum thickening effect the Carbopol molecule must be completely uncoiled which can be achieved by the addition of an appropriate neutralizing agent.[10] HPMC is useful for thickening both aqueous and non-polar gels and frequently used in the concentration of 2–10%.[11] HPMC is known to improve the appearance of skin, hair, and nails[12] and also acts as an anti-aging agent.[13]The careful selection of oil in a microemulsion can manifold the benefits of a formulation. Orange oil contains limonene 94.00% as its principal constituent. It is a natural antiseptic, antioxidant, [14] anti-inflammatory, anti-allergic, and fungicidal. Therefore, orange oil is a suitable for the treatment of various skin conditions such as dermatitis and skin irritation. In addition orange oil also acts as a penetration enhancer which can be due to the presence of terpenes. [15]

Materials and Methods

Materials:

orange oil, and Tween 20, were procured from Research lab fine Chemicals Industry, Mumbai, India. PEG-200 was purchased from Rankem Laboratory Reagent. Mumbai. NA was cordially provided as a gift sample from Ishita Drugs & Industries Ltd. Ahmedabad (Gujrat). HPMC was purchased from Research lab Mumbai Maharashtra, India, and Carbopol 934p was Gift sample from Shin-Etsu ChemicalsCo.Ltd, India. Distilled water was used during the entire experiment. All other chemicals and solvents were of analytical reagent grade.

Methods:

Screening of components: The most important criterion for the screening of components for microemulsion is the solubility of poorly soluble drug in oils, surfactants and Cosurfactants. The solubility of Nicotinamide in various oils, surfactants, Cosurfactants and water was determined by adding an excess amount of drug in 2 mL of selected oils, surfactants, Cosurfactants and distilled water separately in 5 mL capacity stopper vials, and mixed using a ultrasonicator. The mixtures in vials were then kept at room temperature in an rotary shaker for 48 h to reach equilibrium. The equilibrated samples were removed from shaker and centrifuged at 3000 rpm for 15 min. The supernatant was taken and filtered through a 0.45 µm membrane filter. The concentration of Nicotinamide was determined in oils, surfactants, Cosurfactants and water using UV spectrophotometer at

262 nm. For each excipient determine standard calibration curve for determination of concentration of Nicotinamide in excipients (16).

Pseudo Ternary Phase Diagram Studies: On the basis of the solubility studies of drug, select the oil phase, surfactants and Cosurfactants. Water was used as an aqueous phase for the construction of phase diagrams. Surfactant and cosurfactant (Smix) are mixed in different weight ratios 1:0, 1:1, 2:1, 3:1. These Smix ratios were chosen in increasing concentration of surfactant with respect to cosurfactant and increasing concentration of cosurfactant with respect to surfactant for detailed study of the phase diagrams for formulation of microemulsion. For each phase diagram, oil and specific Smix ratio was mixed thoroughly in different weight ratios from 1:9 to 9:1 in different glass vials. Seventeen different combinations of oil and Smix, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1 were made so as to cover possible combinations for the study to delineate the boundaries of phases precisely formed in the phase diagrams. Pseudo ternary phase diagrams were developed using aqueous titration method. Slow titration with aqueous phase was done to each weight ratio of oil and Smix and visually observed for transparent and easily flowable o/w microemulsions. The physical state of the microemulsion was marked on a pseudo-three component phase diagram with one axis representing aqueous phase, the other representing oil and the third representing a mixture of surfactant and cosurfactant at fixed weight ratios (Smix ratio) (16). Based on the results, appropriate percentages of oil, surfactant and co-surfactant were selected and correlated in the phase diagram and then were used for preparation of microemulsion of Nicotinamide.

Preparation of the Nicotinamide loaded microemulsion: Nicotinamide was added to the mixtures of oil and Smix in selected formulation as reported in Table 2, and then an appropriate amount of water was added to the mixture drop by drop and the microemulsion containing Nicotinamide was obtained by stirring the mixtures. All microemulsions were stored at 30 ± 2^{o}C. Nicotinamide at 1.5 %w/w was incorporated in all formulations (14). Compositions of Nicotinamide loaded microemulsions are reported in Table 3. Saturated solution of Nicotinamide as control was prepared by dissolving sufficient Nicotinamide in ethanol (14).

Selection of microemulsion composition: Different compositions of O: W: S were chosen from ternary phase diagram and subjected to stability studies at 5°C (refrigerator), 25°C (room temperature), and 40°C heating cooling cycle. At the end of the study, sample was observed for clarity, phase separation, and colour change.

Preparation of MBG

Microemulsion was converted to gels using HPMC and Carbopol 934P.

HPMC gel

HPMC was uniformly dispersed in a weighed mixture of oil: surfactant and to that mixture water was added slowly with constant stirring. A clear transparent gel was formed. HPMC gel was prepared in a concentration of 3%, 3.1%, and 3.5%

Carbopol gel

Carbopol was uniformly dispersed in oil: surfactant mixture and water was slowly added with gentle stirring to avoid the formation of bubbles. Gel formed was translucent and more viscous than the HPMC gel. Carbopol gels were prepared in the concentration of 1%, 1.1%, and 1.2%.

Evaluation of Microemulsion based gel

pH measurement: The pH of formulated microemulsion based gel was determined using pH meter (Model EQ-610) Labindia, (Mumbai). The electrode was immersed in microemulsion based gel solution and pH was recorded.

Viscosity Determination: The viscosity of microemulsions were measured at 25^0C using a Brookfield Viscometer (LV) with small sample adapter spindle No. T–91, at 50 rpm.

Spreadability

Spreadability of gel is measured in terms of diameter of gel circle produced when gel is placed between two glass plates of definite weight. A weighed quantity (350 mg) of gel is taken on one glass plate and another glass plate is dropped from a distance of 5 cm. The diameter of the circle of spread gel is measured. Table No -10:2 shows the Spreadability of formulations.

It is calculated by using the formula:

$$S=M.L/T$$

Where,

S= Spreadability

M= weight tied to upper slide.

L= length of glass slide.

T= time taken to separate the slides completely

Percentage drug content of the formulation

The percentage drug content of the formulation was analyzed by dissolving 1 gm of the formulation in 10 ml phosphate buffer pH 7.4. After suitable dilutions with ehanol, absorbance was determined using the UV spectrophotometer (UV V-730, Jasco, Japan) keeping blank microemulsion as control at wavelength 262 nm.

Particle size analysis

For the determination of droplet size and zeta potential the prepared formulations were suitably diluted with distilled water. To ensure complete dispersion of the formulation, the samples were inverted twice. Following complete dispersion, the Microemulsions were subjected to **Malvern Panalytical** for the droplet size determination. The principle involved is due to Brownian motion of droplets as a function of time which is determined due to fluctuation in light scattering, and it determines by photon correlation spectroscopy.

Polydispersity Index

Polydispersity Index (Pl) of the prepared microemulsion based gel was determined using Dynamic Light Scattering (DLS) method. For DLS method, the sample needs to be crystal clear to very slightly hazy. If the solution is white or too hazy, it should be diluted further before attempting a DLS size measurement. When the solution was ready for analysis and transfer it in the cuvette, care should be taken to avoid bubbles which are formed on the walls of the cuvette. Slowly tilting or tapping the cuvette on a hard surface may also help to remove the bubbles formed. Once the solution was homogenous and ready for DLS measurement, the cuvette containing the solution can be placed in the instrument. The instrument was run and solution was analysed for Polydispersity Index (PI).

Zeta Potential analysis

Zeta Potential of the prepared microemulsion based gel was determined using Light Scattering method. For Zeta Potential determination, the sample needs to be crystal clear. When the solution was ready for analysis and transfer it in the cuvette, care should be taken to avoid bubbles which are formed on the walls of the cuvette. Slowly tilting or tapping the cuvette on a hard surface may also help to remove the bubble formed. Then the electrode was dipped inside the cuvette containing sample solution. Care should be taken to avoid bubbles in between the electrodes. The cuvette containing the solution can be placed in the instrument. The instrument was run and solution was analysed for Zeta Potential.

***In-Vitro* drug release study**

Franz diffusion cells with a cellulose membrane were utilized to determine the Release rate of Nicotinamide from different microemulsion gel formulations. The cellulose (molecular weight G12 000) membrane was first hydrated in the distilled water solution at 25 °C for 24 hours. The membrane was then clamped between the donor and receptor compartments of the cells Diffusion cell was filled with 25 ml of phosphate buffer (pH = 7.4). The receptor fluid was constantly stirred by externally driven magnetic bars at 600 rpm throughout the experiment. Nicotinamide microemulsion (2 g) was accurately weighted and placed in donor compartment. At 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 24 h time intervals, 1 ml sample was removed from receptor for spectrophotometric determination and replaced immediately with an equal Volume of fresh receptor solution. Samples were analyzed by UV visible spectrophotometer at 262 nm. The results were plotted as released drug percent versus time.

Stability of microemulsion based gel

The prepared MBGs were filled in glass vials and subjected to stability studies at 5°C (refrigerator), 25°C (room temperature), for 1 month. Samples were withdrawn at 15-day time intervals and assessed for physical changes.

RESULT AND DISCUSSION

Screening of Components: The most important criterion for screening of excipients is the solubility of the poorly soluble drug in oil, surfactants, and co-surfactants. Since the aim of this study is to develop a topical

formulation, it is important to determine the solubility of the drug in different oils, surfactants, and co-surfactants. The solubility of Nicotinamide in different oils surfactants, Cosurfactants and water was determined (Table 1). The solubility of Nicotinamide was found to be highest in orange oil (15.8 ±1 .35 mg/mL) and olive oil (14.2 ±2.87mg/ml) as compared to other oils while in Tween 20 which was show highest solubility of Nicotinamide of 18.2 ±2.58mg/ml. PEG-200, Ethanol and show the solubility of Nicotinamide 17.47 ±2.58mg/mL, 15.22 ± 0.07 mg/mL respectively. Orange oil and Tween 20 selected as oil and surfactant respectively. P. Santos et al. reported medium chain triglycerides of caprylic acid and capric acid have been employed as the oil phase in a number of topical microemulsion formulations (19). Because of orange oil is selected as oil phase and Nicotinamide show highest solubility in orange oil. Tween 20 selected as surfactant because HLB value 16.7, Non-ionic surfactants are less toxic than ionic surfactants, good biological acceptance, powerful permeate enhancers and highest solubility of Nicotinamide (20). PEG 200 selected as cosurfactant because highest solubility of Nicotinamide. On the other hand, in ethanol highest solubility of Nicotinamide but alcohols and other volatile co-solvents have the disadvantage of evaporation there may be chances drug precipitation (21).

Table (1): Solubility of Nimodipine in various oils, Surfactant, Cosurfactants at room temperature

Sr.no	Components	Solubility (mg/ml)
1	Orange oil	15.8 ±1.35
2	Olive oil	14.2 ±2.87
3	Peppermint oil	5.52 ±1.68
4	Castor Oil	11.2 ±0.47
5	Span 60	16.6 ±1.54
6	Span 20	5.34 ±3.01
7	Tween 20	18.2 ±2.58
8	PEG-200	17.47 ±2.58
9	Ethanol	21.46 ±3.02
10	Cetyl alcohol	28.52 ±2.88

Pseudo Ternary Phase Diagram Studies:

Constructing phase diagrams is time consuming, particularly when the aim is to accurately delineate a phase boundary. Care was taken to ensure that observations are not made on metastable systems, although the free energy required to form an emulsion is very low, the formation is thermodynamically spontaneous. The relationship between the phase behaviour of a mixture and its composition can be captured with the aid of a phase diagram. Pseudo ternary phase diagrams were constructed separately for different Smix ratios, so that o/w microemulsion regions could be identified and microemulsion formulations could be optimized (16). In

Figure 1 (S mix ratio1:0) it can be observed that when Tween 20 was used alone without cosurfactant, 9.43 % w/w oil could be solubilized at a low concentration 42.18 %w/w of surfactant. As the concentration of surfactant decreased solubilization of oil decreased. When cosurfactant was added with surfactant in equal amount [Smix ratio1:1(Figure 2)], the microemulsion region in the phase diagram decreased and the very low amount of oil 1.01 %w/w could be solubilized at the concentration 12.49 %w/w of surfactant. When cosurfactant concentration was further increased to S mix ratio 2:1 (Figure 3), it was observed that the microemulsions area is a increased as compared to Smix ratio 1:1. 8.5 %w/w oil is solubilized in high concentration of surfactant 37.02 % w/w. When surfactant concentration was increased with respect to cosurfactant [Smix ratio 2:1 (Figure 4)], it was seen that 20 %w/w oil could be solubilized with a surfactant concentration of 57 % w/w. When the surfactant concentration was further increased to 3 parts is to 1 part of cosurfactant (Figure 5), the microemulsion area decreased further and maximum amount of oil that could be solubilized was 10 %w/w and that too at a lower concentration of Smix (21 %w/w). It can be observed that the formulations prepared from phase diagrams in which the microemulsion area was extended towards aqueous rich apex could be diluted to a larger extent. Smix ratio 2:1 shows maximum solubility of oil in minimum surfactant concentration. This Smix ratio select for the preparation of Nicotinamide loaded microemulsion. four formulations selected for preparation of Nicotinamide loaded microemulsion from microemulsion region of Smix ratio 2:1 (Table 2) Also Smix ratio 3:1 is shows maximum solubility of oil in minimum amount of surfactant mixture.

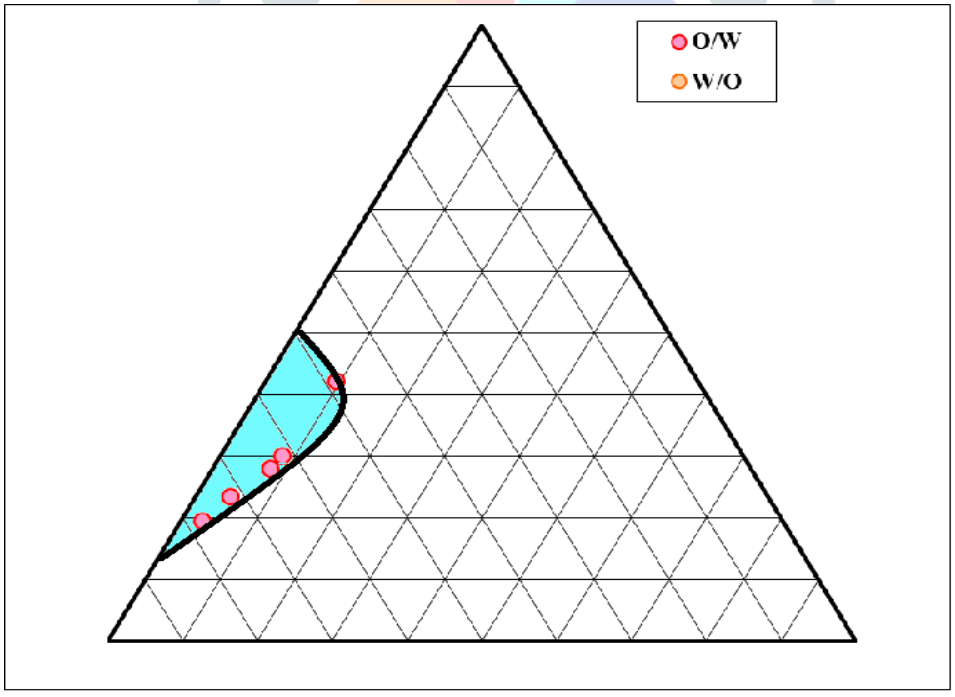

Figure 10.10: A Smix 1:0

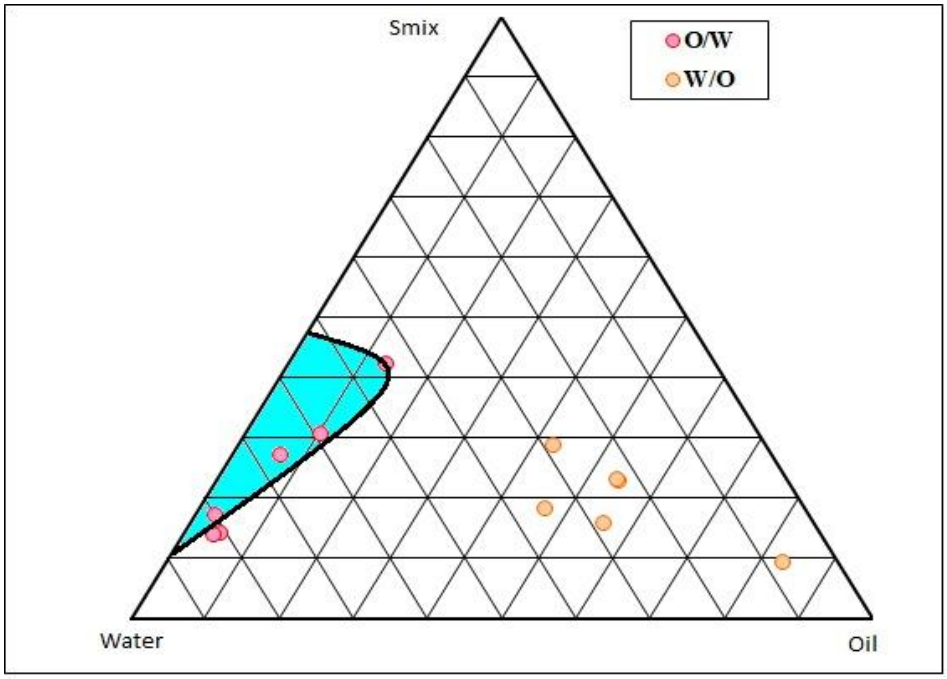

Figure 10.11: B Smix 1:1

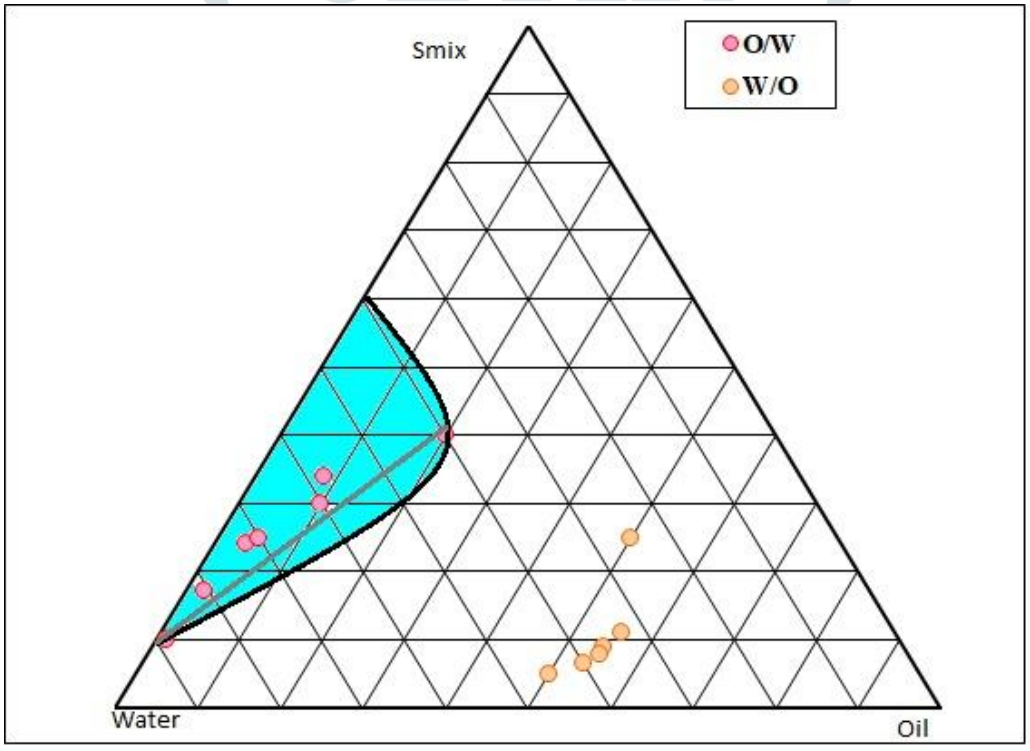

Figure 10.12: C Smix 2:1

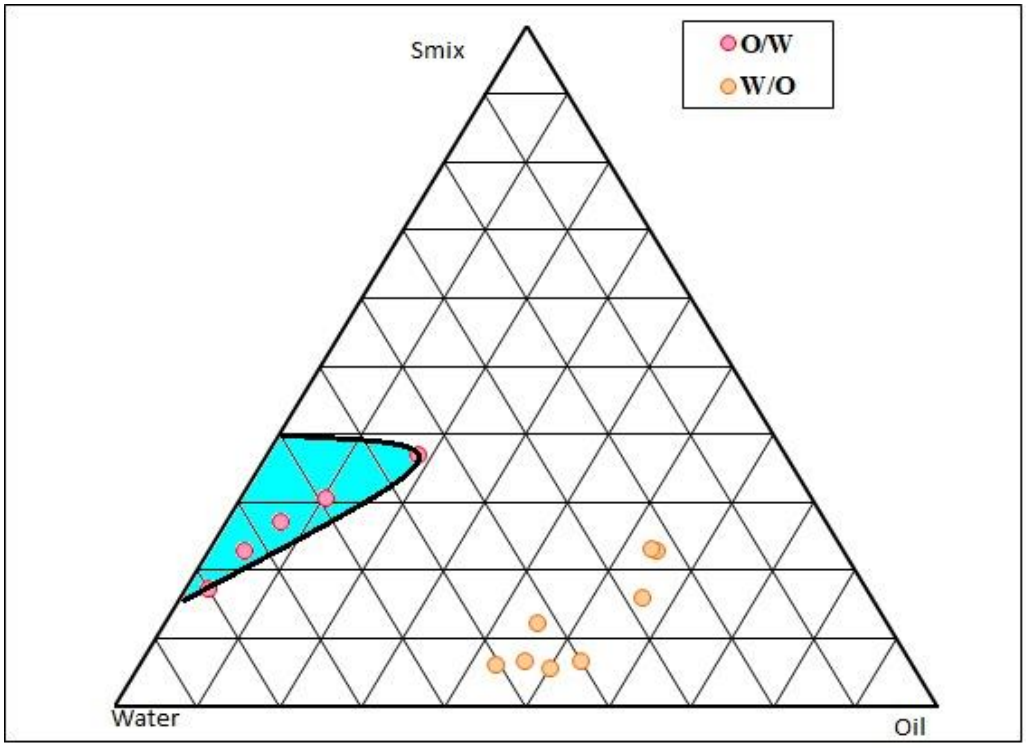

Figure 10.13: D Smix 3:1

Preparation of the Nicotinamide loaded microemulsion

Nicotinamide was added to the mixtures of oil and Smix in selected formulation as reported in Table 2

Table 2: Preparation of the Nicotinamide loaded microemulsion

Sr.NO.	Microemulsion	Nicotinamide (%w/w)	Oil (%w/w)	Smix (%w/w)	Water (%w/w)
1	M2	1.5	5	25	68.34
2	M4	1.5	2.4	17.09	78.98
3	M3	1.5	6.75	27.02	64.71
4	M5	1.5	2.85	17.14	78.5

Preparation of MBG

Microemulsion was converted to gels using Carbopol 934P.

Table 3: Preparation of MBG

Sr.NO.	Microemulsion	Nicotinamide (%w/w)	Oil (%w/w)	Smix (%w/w)	Water (%w/w)	Carbopol (%w/w)
1	MBG 2	1.5	5	25	68.34	1.1
2	MBG 4	1.5	2.4	17.09	78.98	1.1
3	MBG 3	1.5	6.75	27.02	64.71	1.1
4	MBG 5	1.5	2.85	17.14	78.5	1.1
5	Controled gel	1.5	-	-	100	1.1

Evaluation of Microemulsion based gel

pH measurement: The pH of the prepared microemulsion based gel was found to be between 4.8±0.38to 5.68±0.18

Viscosity Determination: The rheological properties of the microemulsion based gel are evaluated by Brookfield Viscometer. The MBG 5 formulation shows the lowest viscosity i.e. 42.36 ±1.54

Spreadability: The Spreadability of microemulsion based gel of different batches was found to be 28.23 to 44.03

Table 4: pH, Viscosity and Spreadability of the MBG

Sr.no.	Batch	pH	Viscosity	Spreadability
1	MBG 2	4.8±0.38	54.44 ±3.89	28.23
2	MBG 3	4.69±0.14	48.35±2.01	38.45
3	MBG 4	5.2±0.42	46.91±1.08	40.50
4	MBG 5	5.68±0.18	42.36±1.54	44.03

Percentage drug content of the formulation: The drug content of all formulations ranged between 95.65 ±0.052 to 98.34±0.021% and passed uniformity of content. The drugs content of MBG 5 formulation was found to be 98.34±0.021 while other formulation drug content found less than 95% so it was concluded that MBG 5 formulation have more drug content as compare to others.

Particle size analysis: The rate and extent of drug release and absorption could be dependent on the globule size, the particle size of MBG 5 was found to be 24.46

Polydispersity Index: Polydispersity Index (PI) of the prepared microemulsion based gel was determined using Dynamic Light Scattering (DLS) method the PI of MBG 5 was found to be 0.368

Zeta Potential analysis: Zeta Potential results for all the prepared batches of Nicotinamide microemulsion based gel are MBG 2, to MBG 5 was found to be 41.05, 58.59, -35, -53.68.

Table 5: Percentage drug content, Particle size, Polydispersity Index, Zeta Potential

Sr.no.	Batch	Percentage drug content	Particle size	Polydispersity Index	Zeta Potential
1	MBG 2	95.65±0.052	123.2	0.724	41.05
2	MBG 3	96.34±0.032	162	0.875	-35.00
3	MBG 4	97±0.036	139	0.501	58.59
4	MBG 5	98.34±0.021	24.46	0.368	-53.68

***In-Vitro* Drug Release Study:** The in-vitro release of NA-Microemulsion based gel was examined for optimized formulation MBG 2, MBG 3, MBG 4 and MBG 5 was performed. It determines that percentage drug release of MBG 5 formulation is 77.38 ±2.98 it is more than MBG 2, MBG 3, MBG 4 formulation. It can be concluded that the developed microemulsion MBG 5 have great potential for Topical drug delivery.

Table 6: *In-Vitro* drug release in 24 hours

Sr.no	Batch	% Drug Release
1.	MBG 2	58.92 ±3.99
2.	MBG 3	43.00 ±4.51
3.	MBG 4	61.26 ±3.95
4.	MBG 5	77.38 ±2.98
5.	Controled	48.00 ±4.51

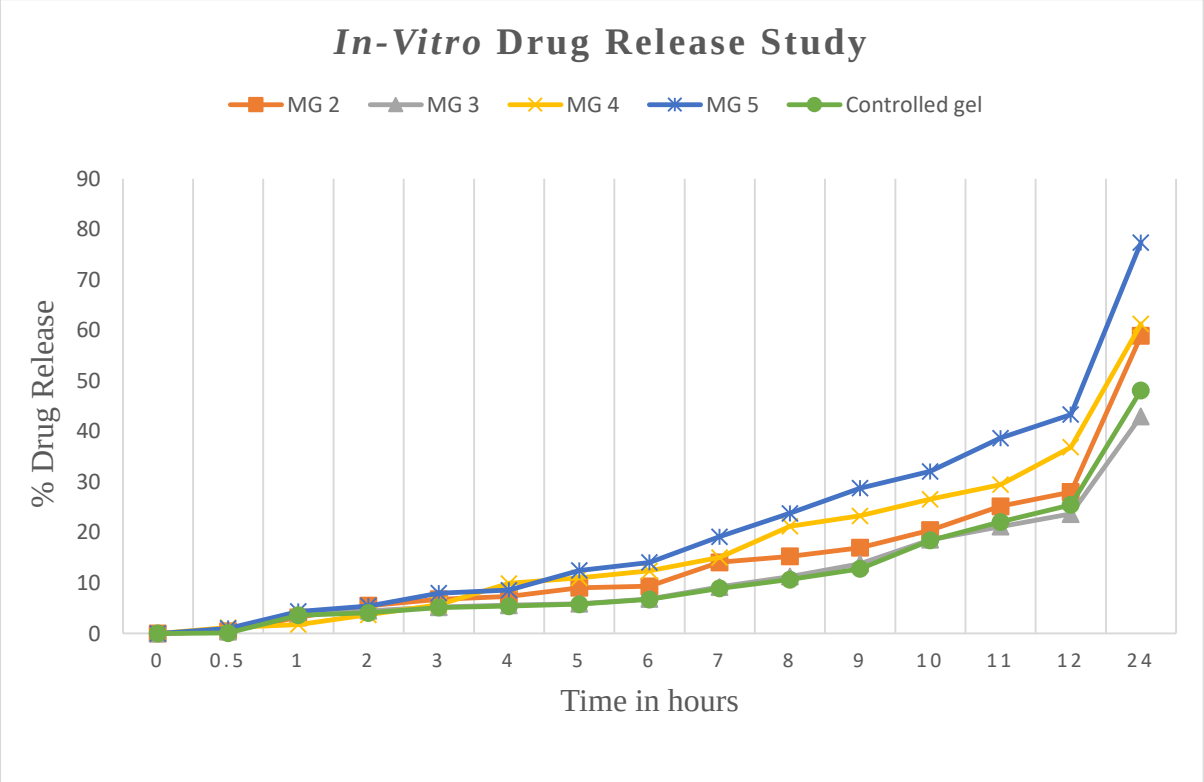

Figure 10.22: *In-Vitro* drug release study

Conclusion

The present study aim was to formulation evaluation and optimization of the Nicotinamide microemulsion based gel to improve the Permeability of drug and bypass the hepatic first pass metabolism on topical application. microemulsion based gel was prepared by the Phase titration method. Five batch were prepared of the microemulsion based gel and from that MBG 5 batch was final and optimize for the stability study. MBG 5 batch particle size was observed 24.46 nm. Also the drug release of the MBG 5 is highest which is

77.38 ±2.98 within 24 Hours. In this present study, Nicotinamide microemulsion based gel were successfully prepared and evaluated for different parameters such as particle size, zeta potential, in- vitro drug release and stability studies. The optimized microemulsion based gel (MBG 5) formulation consist of oil phase (orange oil), surfactant (Tween 20) and cosurfactant (PEG-200) with zeta potential -53.68 mV and particle size 24.46. Stability data indicated stable formulation.

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