

Formulation, Characterization, and *Ex vivo* Evaluation of Microemulsion Based Gel of Nicotinamide

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Abstract

Introduction: Nicotinamide (NA) has shown potential benefits in acne vulgaris, photodamage, cellulite, and atopic dermatitis. **Aim:** In this study, a topical microemulsion based gel (MBG) of NA has been prepared. **Materials and Methods:** Microemulsion was composed of NA, Peppermint oil, Tween 20, and distilled water. The microemulsion was converted into MBGs using various concentrations of hydrophilic colloidal silica and Carbopol 934P. MBGs which were found to be the most stable ones were evaluated for further parameters. **Results:** MBGs with 4.1% hydrophilic colloidal silica and 1.1% Carbopol 934P were observed to be most stable after stability studies and were further assessed for rheology, spreadability, bioadhesion, and *ex-vivo* release and retention. The results connoted that MGB colloidal silica 4.1% was clear and MBG Carbopol 934P 1.1% was translucent, both with shear thinning properties. The *ex vivo* evaluation using pig ear skin was performed for both the gels to study the drug release and drug retention in comparison to NA solution. The release kinetics was fitted to zero-order model. The drug release and retention were higher in the following order MBG Carbopol >MBG Colloidal Silica >NA solution. **Conclusion:** The study indicates that MBG can be an effective system for topical delivery of NA.

Key words: Acne vulgaris, Microemulsion based gel, Microemulsion, Nicotinamide

INTRODUCTION

Acne is a disorder of the pilosebaceous unit comprising abnormalities in sebum production and characterized by both inflammatory (papules, pustules, and nodules) and non-inflammatory (comedones, open, and closed) lesions. *Propionibacterium acnes* and *Staphylococcus epidermidis* are the common pus-forming microbes that are responsible for the development of various forms of acne vulgaris.^[1]

Nicotinamide (NA) is also known as Vitamin B3, vitamin pp, or nicotinic acid amide. NA acts as an anti-acne agent due to its anti-inflammatory action and also inhibits *P. acnes*.^[2] It is used both topically and orally for the treatment of mild to moderate acne. NA also improves 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 acne blemishes and scars. On aging skin, NA improves the surface structure of the

skin, shows a wrinkle smoothing effect and also inhibits photocarcinogenesis.^[5,6]

Shalita *et al.* reported that 4% NA gel is of comparable efficacy to 1% clindamycin gel in the treatment of acne vulgaris. As topical clindamycin, like other antimicrobials, is associated with the emergence of resistant microorganisms, NA gel can be a desirable alternative treatment for acne vulgaris.^[7]

Microemulsions have been previously used in the treatment of acne,^[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

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of the hydrophilic and lipophilic moiety of the surfactant interface film.^[9] Therefore, microemulsion can be a favorable 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.

Carbopols are the most commonly used gelling agents in the pharmaceutical and cosmetic industry. To attain maximum thickening effect the carbomer molecule must be completely uncoiled which can be achieved by the addition of an appropriate neutralizing agent.^[10] Colloidal silicon dioxide is useful for thickening both aqueous and non-polar gels and frequently used in the concentration of 2–10%.^[11] Colloidal silica 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. Peppermint oil contains menthol (33–60%) as its principal constituent. It is a natural antiseptic, antioxidant,^[14] anti-inflammatory, anti-allergic, and fungicidal. Therefore, peppermint oil is a suitable for the treatment of various skin conditions such as acne and skin irritation. In addition, peppermint oil also acts as a penetration enhancer which can be due to the presence of terpenes.^[15]

MATERIALS AND METHODS

Materials

Peppermint oil was purchased from Molychem. Tween 20, Tween 80, and oleic acid were procured from Loba Chemie, Mumbai, India. Soybean oil was food grade. NA was cordially provided as a gift from Vasu Enterprises Pvt., Ltd., Ludhiana, India. Hydrophilic colloidal silica (Aerosil 200) was provided as a gift sample from Evonik Industries, Maharashtra, India,

and Carbomer was purchased from Central Drug House, India. Distilled water was used during the entire experiment. All materials were used as received.

Methods

Screening of oils and surfactants for microemulsion

The solubility of NA was evaluated in various oils, surfactants, and water. To determine the solubility excess amount of NA was dispersed containing 1 g of each component in glass vials. The mixture was then vortexed for 5 min to facilitate proper mixing of NA with the components taken in the glass vials and kept under shaking for 48 h in a water bath shaker maintained at $25 \pm 2^\circ\text{C}$ followed by centrifugation at 5000 rpm for 10 min.^[16] The supernatant was then withdrawn and was quantified after appropriate dilution using ultraviolet (UV) spectrophotometry at λ_{max} of 262 nm (λ_{max} of NA). Appropriate dilutions of oils and surfactants were taken as blank, and each experiment was performed in triplicate, and the average value was determined.

Formulation of microemulsion

Construction of ternary phase diagram

Ternary phase diagram was constructed using aqueous titration method to obtain concentration range of microemulsions giving single phase region with different possible compositions of oil, surfactant, and water. The oil was mixed with surfactant to prepare weight ratios of oil:surfactant: 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, and 10:90. The oil and surfactant mixtures were magnetically stirred at 37°C for few minutes to facilitate the mixing process. Then, distilled water was added drop by drop to the oil:surfactant mixture. The endpoint of the titration was the point where the mixture becomes turbid and the amount of aqueous phase sufficient to cause turbidity was recorded. The amount of the aqueous phase sufficient to cause turbidity was recorded.^[17]

Preparation of microemulsion by water titration method

The transparent and single phase mixtures were determined as ideal microemulsions. Using ternary phase diagram appropriate composition of oil, surfactant and water, and forming single phase microemulsion was selected and tested for stability. The stable microemulsion composition was used in the preparation

Table 1: Solubility values for different excipient

Solvent	Solubility (g/ml)
Peppermint oil	0.121
Soybean oil	0.012
Oleic acid	0.095
Tween 80	0.101
Tween 20	0.289
Water	0.600

Table 2: Composition of NA microemulsions

Formulations	Composition	Clarity	Color	Phase separation
O:T:W, F1	30%: 46.15%: 23.07%	Clear, transparent	Yellow	No
O:T:W, F2	28.57%: 42.85%: 28.57%	Clear, transparent	Yellow	Yes
O:T:W, F3	33%: 50%, 17%	Clear, transparent	Yellow	Yes

NA: Nicotinamide

Table 3: Composition of MBGs

MBG colloidal silica	MBG Carbopol
Colloidal silica 3.6%	Carbopol gel 1% 30 g
Oil 11.15	Oil 11.50
Tween 20 12.6	Tween 20 13
Water 5.73	Water 5.88
NA 1.2	NA 1.2
Colloidal silica 1.08	Carbopol 0.30
Colloidal silica 4.1%	Carbopol gel 1.1% 30 g
Oil 10.39	Oil 10.74
Tween 20 11.80	Tween 20 12.19
Water 5.34	Water 5.52
NA 1.2	NA 1.2
Colloidal silica 1.23	Carbopol 0.33
Colloidal silica 4.6%	Carbopol gel 1.2% 30 g
Oil 10.33	Oil 10.68
Tween 20 11.73	Tween 20 12.13
Water 5.31	Water 5.49
NA 1.2	NA 1.2
Colloidal silica 1.38	Carbopol 0.36

MBG: Microemulsion based gel, NA: Nicotinamide

Table 4: Results from stability study of gels

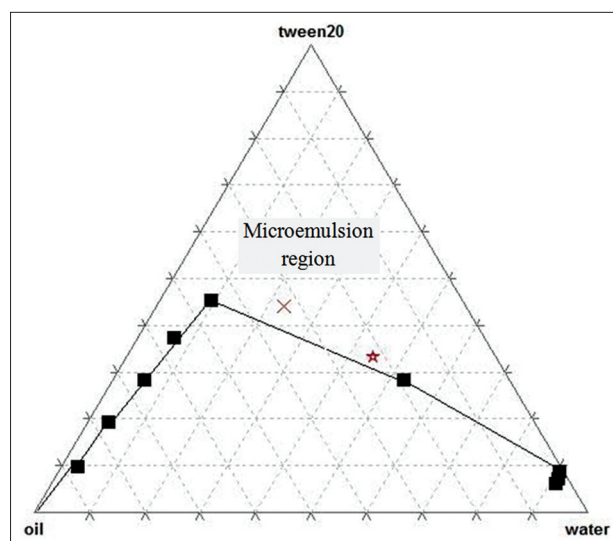
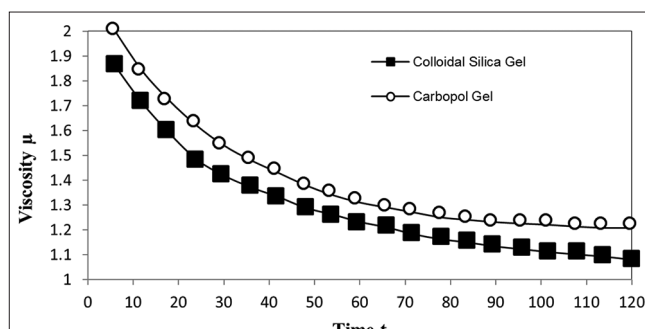
Test	Carbopol 1%	Carbopol 1.1%	Carbopol 1.2%
Syneresis	Yes	No	No
Consistency	Fine	Good	Thick
	Colloidal silica 3.6%	Colloidal silica 4.1%	Colloidal silica 4.6%
Syneresis	Yes	No	No
Consistency	Fine	Good	Thick

Table 5: Drug content measurement values for MBG

Sample	Drug content (%)
Colloidal silica gel	
Sample 1	98.9206
Sample 2	97.8728
Sample 3	99.8729
Carbopol gel	
Sample 1	98.7379
Sample 2	98.3863
Sample 3	99.4400

MBG: Microemulsion based gel

of microemulsion based gel (MBG). No attempts were made to determine the type of microemulsion formed.


Figure 1: Ternary phase diagram for peppermint oil, tween 20, and water

Figure 2: Rheology curve for colloidal silica gel and Carbopol gel

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/75% RH for 1 month. At the end of the study, sample was observed for clarity, phase separation, and color change.

Preparation of MBGs

Microemulsion was converted to gels using hydrophilic colloidal silica and Carbopol 934P.

Colloidal silica gel

Colloidal silica 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. Colloidal silica gel was prepared in a concentration of 3.6%, 4.1%, and 4.6%.

Carbopol gel

Carbopol was uniformly dispersed in oil:surfactant mixture and warm water was slowly added with gentle stirring to avoid the formation of bubbles. Gel formed was translucent

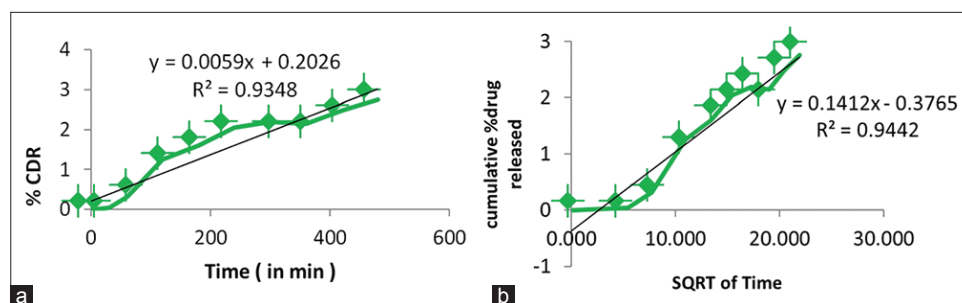


Figure 3: Cumulative drug release profile for Nicotinamide solution (a) zero order plot and (b) Higuchi Plot

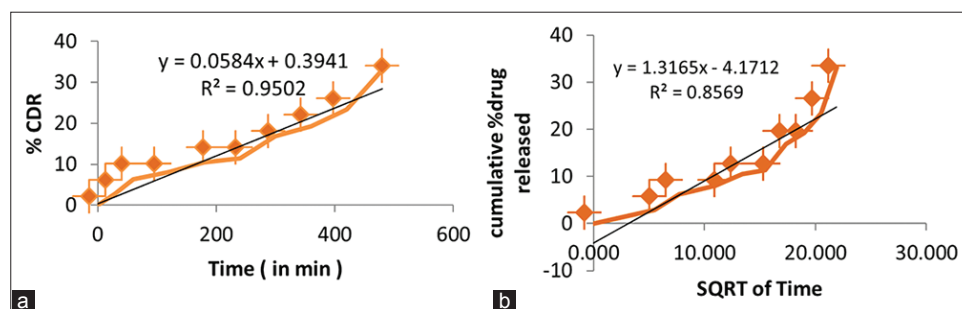


Figure 4: Cumulative drug release profile for Carbopol gel (a) zero-order plot and (b) Higuchi Plot

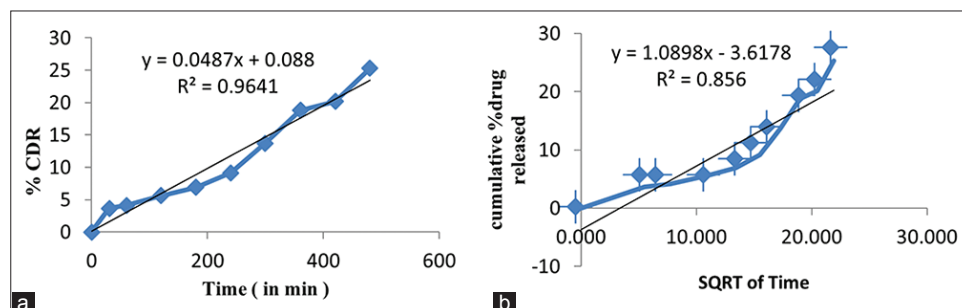


Figure 5: Cumulative drug release profile for colloidal silica gel (a) zero-order plot and (b) Higuchi Plot

and more viscous than the colloidal silica gel. Carbopol gels were prepared in the concentration of 1%, 1.1%, and 1.2%.

Characterization of Gel

Stability studies of MBGs

The prepared MBGs were filled in glass vials and subjected to stability studies at 5°C (refrigerator), 25°C (room temperature), and 40°C/75% RH for 1 month. Samples were withdrawn at 15-day time intervals and assessed for physical changes.

Physical changes

Physical changes in samples such as phase separation and clarity were observed.

The most stable MBG was further characterized by the following parameters:

Viscosity

Viscosity was measured using Anton Paar Rheolab QC. The determinations were performed in triplicate at 32°C, imitating the temperature of human skin.

Drug content determination

The drug content in the gellified emulsion was measured by dissolving 100 mg of gel in 10 ml of water by sonication. Absorbance was measured after suitable dilutions in UV/VIS spectrophotometer. The experiment was performed in triplicate.

Spreadability study

Spreading coefficient was determined using apparatus as suggested by Mutimer. It consists of a wooden block, which is attached to a pulley at one end. Spreading coefficient was measured by utilizing “Slip” and “Drag” characteristics of gels. A ground glass slide was fixed on the wooden block. An excess of gel (about 1 g) under study was placed on

this ground slide. The gel preparation was then sandwiched between this slide and second glass slide having the same dimension as that of the fixed ground slide. The second glass slide was provided with the hook. The weight of 500 mg was placed on the top of the two slides for 5 min to expel air and to provide a uniform film of the gel between the two slides. The measured amount of weight was placed in the pan attached to the pulley with the help of hook. The time (in seconds) needed by the top slide to cover a distance of 7.5 cm was noted. A shorter interval indicates better spreading coefficient.^[18]

Bioadhesion testing

The modified method was used for the measurement of bioadhesive strength. The apparatus consists of two arm balance. Both the ends are tied to glass plates using strings. One side contains two glass plates. Another side comprises a single glass plate for keeping weight. The right and left pans were balanced by adding extra weight to the left hand pan. The balance was kept in this state for 5 min. Accurately weighed 1 g of MBG was placed between these two slides containing hairless fresh pig ear skin pieces, and extra weight from the left pan was removed to sandwich the two pieces of glass and some pressure was applied to exclude the presence of air. The balance was kept in this state for 5 min. Weight was added gradually at 100 mg/min to the left hand pan until the two glass slides got detached from each other. The weight (gram force) needed to detach the two slides gives the measure of bioadhesive strength (Choi *et al.*, 2003). The bioadhesive strength was calculated using following equation.^[19]:

$$B_s = Wg/A$$

Bs: Bioadhesive strength, Wg: Weight required (in g) and A: Area of skin (in cm²).

Ex vivo evaluation of MBGs

Ex vivo permeation/retention studies of optimized formulation of NA

Porcine ear skin was used for *ex vivo* studies. The hair of skin was carefully shaved with electrical clippers, and the full thickness skin was removed. The dermal surface of the skin was cleaned to remove subcutaneous tissues and fats without damaging the epidermal surface. The skin was then washed with water and used for *ex vivo* permeation/retention studies.^[18]

Ex vivo permeation studies

The pig ear skin was mounted between the donor and receptor compartments of diffusion cell with stratum corneum facing the donor compartment. MBG enough to cover skin surface was placed in the donor compartment, and 26 mL of phosphate buffer saline was used as receptor medium. The contents of the receptor compartment were agitated at 100 rpm over a multi-magnetic stirrer (Cintex, Mumbai, India). The study was conducted at 37°C, and 1mL samples were collected at predetermined time points. The receptor compartment was replenished with PBS after each sample withdrawal. The cumulative amount of NA permeated was determined using UV at λ_{\max} 262 nm after suitable dilutions and concentration was corrected for sampling effects.^[18]

Ex vivo retention studies

After 9 h of permeation study, the effective diffusion area of the skin was separated, washed with distilled water to remove formulation excess, and then cut into small pieces. The samples were vortexed with water and then left soaked for 24 h to ensure efficient extraction of the retained drug

Table 6: Visual appearance of MBG

Sample	Appearance
Colloidal silica gel	Clear transparent gel
Carbopol Gel	Translucent Gel

MBG: Microemulsion based gel

Table 7: Viscosity values for MBGs

Sample	Viscosity (Pa)
Colloidal silica gel	1.2945±0.219
Carbopol gel	1.4007±0.231

MBG: Microemulsion based gel

Table 8: Spreadability measurement values for MBGs

Sample	Spreadability
Colloidal Silica Gel	30.12 g.cm/s
Carbopol Gel	44.03 g.cm/s

MBG: Microemulsion based gel

Table 9: Bioadhesion measurement values for MBGs

Sample	Bioadhesion value (g/cm ²)
Colloidal silica gel	0.792±0.0092
Carbopol gel	1.009±0.0058

MBG: Microemulsion based gel

Table 10: Retention values for different formulations

Formulation	% Retention of NA in Skin
Colloidal silica gel	30.9547
Carbopol gel	32.3814
NA solution	10.6466

NA: Nicotinamide

from the skin. The skin pieces were crushed, and extraction process was repeated to ensure complete extraction of NA. The resulting mixture was filtered using 0.45 μ filter, and suitable dilutions were prepared using PBS. The dilutions were then spectroscopically analyzed at 262 nm.^[20]

RESULTS AND DISCUSSION

Screening of oils and surfactants for microemulsion

The solubility of NA was analyzed in various oils and surfactants. Peppermint oil was chosen as oil phase and tween 20 as the surfactant due to the highest solubility of NA in both of them [Table 1].

Formulation of microemulsion

Construction of ternary phase diagram

Ternary phase diagrams were constructed to determine the optimum concentration of oil, surfactant, and water with the help of Triplot software, version 4.1.2. Microemulsion region or the clear region was observed in the upper portion of the phase diagram whereas the lower region was turbid [Figure 1].

Selection of microemulsion for the preparation of MBG

Three microemulsions compositions were chosen from ternary phase diagram and were subjected to stability study and were observed from different parameters [Table 2]. At the end of the study F1 was chosen for the formulation of MBG.

Preparation of microemulsion gels

Microemulsions improve drug permeation through the skin, but due to low viscosity making retention time too short; therefore, they should be converted to a gel. The MBGs were prepared using Carbopol 934P and colloidal silica in the following compositions [Table 3]:

Test for Microemulsion gels

Stability of MBGs

All the prepared compositions of MBGs were subjected to stability studies for 1 month at room temperature (25°C), refrigerator (2–8°C), and 40°C/75% RH. It was discerned that MBG Carbopol 1.1% and colloidal silica 4.1% were the most stable ones and these were selected for further evaluations [Table 4].^[21]

Drug content determination

The experiment was done in triplicate for both the preparations and following values were obtained for drug content [Table 5]:

Appearance

The appearance of MBGs was optically observed, and following observations were made [Table 6].

Viscosity

For rheological evaluation, colloidal silica gel 4.1% and carbomer gel 1.1% were used. The rheology curves reflect that both the gels were shear thinning systems [Figure 2 and Table 7].

Spreadability study

The spreadability values indicate that Carbopol gel has better spreadability. At the end of the study, following values were obtained [Table 8]:

Bioadhesion testing

The bioadhesive strength was calculated using following formula:

$$B_s = W_g/A$$

Where B_s is bioadhesive strength, W_g is weight required (in gm), and A is area (in cm^2). Following results were obtained [Table 9]:

Ex vivo evaluation of MBGs

Ex vivo permeation studies

Ex vivo permeation studies were performed to compare the cumulative drug permeation from the MBG containing colloidal silica 4.1 % [Figure 5] and Carbopol 1.1% [Figure 4] with NA solution [Figure 3]. The release characteristics were observed in zero-order model and Higuchi model. It was observed that Carbopol gel has better release than colloidal silica gel and both the gels were best fitted to zero-order model.^[22]

Ex vivo retention studies of optimized formulation of NA

The Carbopol gel demonstrated better retention of NA in the skin than colloidal silica gel [Table 10].

CONCLUSION

In the present study, MBG of NA was prepared. The ternary phase diagrams were used to optimize the oil, surfactant, and water ratio. The prepared microemulsion was clear transparent with yellow color. It is necessary for a gel to

give optimized release through the skin which was observed better with Carbopol gel. Clarity was better with the colloidal silica gel, and it was nearly transparent whereas Carbopol gel was translucent. The release kinetics of both the gels was best fitted to zero-order model. The drug retention and drug release were higher in the following order: MBG Carbopol > MBG Colloidal Silica > NA solution. The results suggest that MBGs can be an efficient system for topical delivery of NA.

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