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Original Article

Development and Characterization of Honey-containing Nanoemulsion for Topical Delivery

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Abstract

Background and objectives: Honey is a viscous, hygroscopic liquid in nature. It has the ability to treat wounds, wrinkles, aging, and inflammation. This study's objective was to create and characterize a nanoemulsion containing honey and evaluate its stability.

Methods: A pseudo-ternary phase diagram was retraced with several concentrations of the S_{mix}, water, and liquid paraffin oil to formulate nanoemulsions containing honey. From the results of pre-formulation stability studies, formulation HNE-19, with a hydrophilic lipophilic balance value of 10, and a surfactant and oil ratio of 1:1, was selected as the most stable formulation. HNE-19 and base (B-19) were further subjected to thermodynamic studies of heating and cooling cycles and centrifugation. HNE-19 and its respective base B-19 were characterized for physical changes, droplet size analysis, pH measurements, turbidity, viscosity, and rheological parameters for a period of 90 days.

Results: Results showed that the nanoemulsion containing honey was clear and milky white. There was no evidence of phase separation in HNE-19 and B-19 after the thermodynamic study. The droplet size of fresh HNE-19 was 91.07 nm with a zeta potential of −38.5 mV. After three months, the droplet size and zeta potential were 197.06 nm and −32.5 mV respectively. The observed pH was between 5.8 and 6.7, which corresponds with the pH of the skin. HNE-19 showed non-Newtonian flow and pseudo-plastic behaviour.

Conclusions: Stability and characterization showed that the nanoemulsion containing honey is a remarkable topical delivery formulation and could be evaluated comparatively with conventional topical applications against skin-related diseases like wounds, wrinkles, aging, and inflammations.

Introduction

Honey is categorized as an emollient (softening), humectant (preserving moisture), or moisturizing substance. Since the oldest civilizations, honey has been applied directly to the skin to treat a range of skin disorders. These conditions include diaper dermatitis (an inflammatory reaction of the skin of perianal areas), psoriasis (dry skin lesions), hemorrhoids (lumps inside and around anus), tinea (ringworm), seborrhoea (excessive discharge of sebum), dandruff, and anal fissures (cracks). Additionally, honey has been used as a binder or carrier, an antibacterial, a wound and burn dressing, and as a treatment for pityriasis (rashes). It has emollient, humectant, calming, and hair conditioning benefits in cosmetic compositions. It also maintains youthful skin and delays the appearance of wrinkles, balances pH, and guards against pathogen infections.[1] Lip balms, cleaning milk, moisturizing creams, tonic lotions, after-sun lotions, shampoos, and conditioners are some cosmetics made using honey. The concentrations of honey are typically between 1 and 10 percent, however by combining with oils, gels, emulsifiers, or polymer entrapment, concentrations of up to 70 percent can be

Keywords: Nanoemulsion; Honey; Topical delivery; Skin-related diseases; Inflammation; Wound.

Abbreviations: B, base; B-19, base formulation without honey; HLB, hydrophilic lipophilic balance; HNE, honey containing nanoemulsion; HNE-1 to HNE-33, formulation codes of different honey loaded nanoemulsions; HNE-19, active formulation of honey; mV, milli volt; NE, nanoemulsion; nm, nanometer; O/W, oil in water; RH, relative humidity; S_{mix}, surfactant mixture; W, white.

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achieved.[2]

Nanoemulsions, often referred to as mini-emulsions (submicron), are thermodynamically stable non-equilibrium systems, which in contrast to microemulsions, possess extremely low interfacial tensions. Because of this, and because they lack the droplet flocculation and coalescence that are typically associated with microemulsions, they are sometimes referred to as "approaching thermodynamic stability".[3] The presence of nanosized droplets would create vast interfacial regions that affect the drug's transport characteristics, which are crucial for sustained and targeted drug administration.[4] In comparison to straightforward micellar solutions, these systems have a higher solubilization capacity, and their long-term physical stability gives them an edge over unstable dispersions. Other benefits of nanoemulsions are their ease of manufacture, ability to preserve pharmaceuticals from hydrolysis and enzymatic degradation in physiological conditions, and ability to increase solubility and improve mucosal permeability of weakly water-soluble substances.[3]

Honey's physical characteristics make it difficult to apply directly to an area that is affected because it may liquefy at skin burn temperature and become more fluid at higher temperatures. This issue of liquefaction and leakage, limits the areas of the body where honey can be applied and also makes it challenging to maintain the necessary therapeutic concentration for an extended period. Due to its stickiness, thinness, and liquefaction, honey applied to the skin alone is rarely used in the beauty industry.[5] Also, the hygroscopic nature of honey becomes a problem during processing and storage. The foaming properties of honey are also caused by surface tension and high viscosity.[6]

To the best of our knowledge, we are the first to explore honey in nanoemulsion in order to take advantage of the benefits and avoid the difficulties associated with honey over other dosage forms for cosmetic use. Performance is anticipated to improve when utilizing a lipid-based nanocarrier because interest in nanoscale emulsion has increased significantly in the last few decades due to its unique characteristics, such as elevated stability, aesthetics, and drug delivery qualities. Honey contains a variety of nutrients, mostly fructose and glucose, as well as proteins, amino acids, vitamins, enzymes, minerals, and other insignificant substances that are difficult to absorb.[2] This study aimed to develop a stable honey-based nanoemulsion as an improved topical delivery formulation.

Materials and methods

Honey (Young Natural Honey, which Australian bees collect from farms in the picturesque valleys of Pakistan; Young Natural Honey is a 100 percent pure form of honey), liquid paraffin oil (Hubei, China), Tween 80 (Arshine Pharmaceutical, China), Span 80 (Guangdong, China).

Construction of pseudo-ternary phase diagrams

By means of the software Chemix School 360, a pseudo-ternary phase diagram was constructed, different concentrations of water, surfactant mix (S_{mix}), and liquid paraffin oil were combined (Table 1), and the region of the nanoemulsion was defined. In the preparation of the nanoemulsion (O/W), the pseudo-ternary phase diagram was very important for finding the optimal concentration of the ingredients. From the pseudo-ternary phase diagram, a total of 33 formulations were developed with HLB values 9, 10, 11, and 12. The value of HLB was maintained over 8; for O/W nanoemulsions, the HLB value of S_{mix} is from 8 to 18.

Preparation of honey-loaded nanoemulsion

To identify the constituents and the ranges of their concentrations that can lead to the production of nanoemulsions (NEs), pseudo-ternary phase diagrams were built. 33 formulations were created using this technique (Table 1). A modified method was adopted to formulate honey-loaded nanoemulsions. S_{mix} was dissolved in the liquid paraffin with constant stirring to form an oily phase. The water phase was formed by dissolving honey in water with continuous stirring.[7] The oily phase was added drop by drop to the aqueous or water phase with continuous agitation on a magnetic stirrer. The homogenized mixture (coarse emulsion) was placed in a high-pressure homogenizer (HG-15D, Daihan Scientific Co., Ltd., Korea) at 15,000 rpm for 10 minutes. Homogenization was repeated several times until the nanoemulsion was prepared.

Preliminary stability experiment

All 33 formulations were subjected to a preliminary stability experiment at 25°C for 28 days. Among the total 33 formulations, two formulations, i.e., HNE-18 and HNE-19, were found stable after the preliminary stability study of 28 days.

Long-term stability experiment

HNE-18 and HNE-19 were subjected to characterization and a long-term stability experiment for 90 days at 25°C in tightly closed glass containers. HNE-19 (HLB value 10) was selected as the most stable nanoemulsion at the end of 90 days (Table 2). A base formulation, Base-B19 (without honey), was also prepared using the same procedure for HNE-19.

Thermodynamic stability experiments

HNE-19 and base-B19 were further subjected to the thermodynamic stability experiments of heating-cooling cycle and centrifugation.

Heating Cooling Cycle

HNE-19 and B-19 were subjected to six cycles between 4°C and 45°C for 48 hours in each cycle.[8]

Centrifugation

HNE-19 and B-19 were centrifuged at 3,500 rpm for 30 minutes.[9]

Characterization of HNE-19 and B-19

Physical changes

HNE-19 and B-19 were evaluated for physical changes, i.e., color, odour, liquefaction, and phase separation at various storage temperatures (8°C, 25°C, 40°C, and 40°C with 75% RH) and at different intervals for 90 days.

Size of the droplet, zeta potential, electrical conductivity, mobility and polydispersity

B-19 and HNE-19's average droplet size, zeta potential, electrical conductivity, mobility, and polydispersity were acquired using Zetasizer, NanoZS, and Malvern according to the manufacturer's standard protocols.[10]

pH measurements

pH measurements of the B-19 and HNE-19 were carried out at different time intervals during the 90-day study period after placing

Table 1. Formulations (100% w/w) based on pseudo-ternary phase diagram

F S/No	HNE	Oily phase	Aqueous phase		S_{mix}	
	F code	Paraffin oil	Water	Honey	Tween 80	Span 80
1	HNE-1	20	q.r	5	1.31	1.69
2	HNE-2	20	–	5	1.75	2.25
3	HNE-3	20	–	5	2.19	2.81
4	HNE-4	20	–	5	2.63	3.37
5	HNE-5	20	–	5	3.07	3.93
6	HNE-6	20	–	5	3.51	4.49
7	HNE-7	20	–	5	1.59	1.41
8	HNE-8	20	–	5	2.13	1.87
9	HNE-9	20	–	5	2.66	2.34
10	HNE-10	20	–	5	53.19	2.81
11	HNE-11	20	–	5	3.72	3.28
12	HNE-12	20	–	5	4.26	3.74
13	HNE-13	20	–	5	1.87	1.13
14	HNE-14	20	–	5	2.50	1.50
15	HNE-15	20	–	5	3.13	1.87
16	HNE-16	20	–	5	3.75	2.25
17	HNE-17	20	–	5	4.38	2.62
18	HNE-18	20	–	5	5.00	3.00
19	HNE-19	8	–	5	4.26	3.74
20	HNE-20	16	–	5	4.26	3.74
21	HNE-21	24	–	5	4.26	3.74
22	HNE-22	32	–	5	4.26	3.74
23	HNE-23	40	–	5	4.26	3.74
24	HNE-24	8	–	5	5.00	3.00
25	HNE-25	16	–	5	5.00	3.00
26	HNE-26	24	–	5	5.00	3.00
27	HNE-27	32	–	5	5.00	3.00
28	HNE-28	40	–	5	5.00	3.00
29	HNE-29	8	–	5	5.75	2.25
30	HNE-30	16	–	5	5.75	2.25
31	HNE-31	24	–	5	5.75	2.25
32	HNE-32	32	–	5	5.75	2.25
33	HNE-33	40	–	5	5.75	2.25

HNE-1 to HNE-33, formulation codes of different honey loaded nanoemulsions; q.r, quantity required; S_{mix}, surfactant mixture.

Table 2. Stability of HNE-18 and HNE-19 for 90-day testing period

F S/No	HNE code	Effects at 25°C, After		
		30 days	60 days	90 days
1	HNE-18	Stable	stable	Phase separation
2	HNE-19	Stable	Stable	Stable

HNE-18, honey containing nanoemulsion (code 18), HNE-19, honey containing nanoemulsion (code 19).

them at 8°C, 25°C, 40°C, 40°C with 75% RH. pH measurements were performed using a digital pH meter (model Zubehorbox pH, inoLab Germany). A glass electrode was used for all measurements, and a probe connected to a pH meter was used to record the temperatures of the B-19 and HNE-19. Commercially available buffer tablets (Merck) with pH values of 4.0 and 7.0 were dissolved in 100 mL of distilled water to calibrate the instrument. The glass electrode was immersed directly into the B-19 and HNE-19 for a few seconds to equilibrate.

Table 3. Results of preliminary stability of formulations for 28-day testing period

S/No	HNE code	Changes at 25°C after 7, 14, 21, and 28 days				Fresh pH
		7	14	21	28	
1	HNE-1	Phase separation	–	–	–	6.54
2	HNE-2	Phase separation	–	–	–	6.51
3	HNE-3	Phase separation	–	–	–	6.86
4	HNE-4	Phase separation	–	–	–	6.30
5	HNE-5	Phase separation	–	–	–	6.45
6	HNE-6	Phase separation	–	–	–	6.55
7	HNE-7	Phase separation	–	–	–	6.50
8	HNE-8	stable	Phase separation	–	–	6.62
9	HNE-9	Phase separation	–	–	–	6.77
10	HNE-10	stable	Stable	Liquefaction	–	6.84
11	HNE-11	Phase separation	–	–	–	6.94
12	HNE-12	Liquefaction	–	–	–	6.85
13	HNE-13	Liquefaction	–	–	–	6.74
14	HNE-14	Liquefaction	–	–	–	6.89
15	HNE-15	stable	Stable	Phase separation	–	6.43
16	HNE-16	stable	Liquefaction	–	–	6.98
17	HNE-17	stable	Stable	Phase separation	–	6.11
18	HNE-18	stable	Stable	stable	Phase separation	6.91
19	HNE-19	Stable	Stable	stable	stable	6.70
20	HNE-20	Stable	Stable	Liquefaction	–	6.03
21	HNE-21	Stable	Phase separation	Fungal growth	–	6.53
22	HNE-22	Phase separation	–	–	–	6.40
23	HNE-23	Stable	Phase separation	–	–	6.92
24	HNE-24	Stable	Phase separation	–	–	6.56
25	HNE-25	Stable	Stable	Liquefaction	–	6.33
26	HNE-26	Phase separation	–	–	–	6.55
27	HNE-27	Phase separation	–	–	–	6.98
28	HNE-28	Phase separation	–	–	–	6.02
29	HNE-29	Stable	Stable	Phase separation	–	6.07
30	HNE-30	Liquefaction	–	–	–	6.36
31	HNE-31	Stable	Phase separation		–	6.61
32	HNE-32	Phase separation	–	–	–	6.77
33	HNE-33	Phase separation	–	–	–	6.62

HNE-1 to HNE-33, formulation codes of different honey loaded nanoemulsions.

Turbidity

The turbidity of the formulated nanoemulsion containing honey and its respective base was evaluated visually at different time intervals for 90 days.

Rheological analysis

Rheological measurements of B-19 and HNE-19 were made using the rheometer (Model: DV-III, UK) CP41 at 10–100 rpm speed with 10 increments. Rheological parameters for B-19 and HNE-19 were calculated and analyzed using Rheocalc version 2.6.[11]

Power's Law formula used as:

$$\tau = kD^n$$

where,τ = shear stress, D = yield stress (stress at zero shear rate), k = plastic viscosity, n = shear rate.

The calculated parameters for this model are: Flow index (no units), consistency index (m.P.s), confidence of fit (%).

Results

Preliminary stability

Results of the preliminary stability for the 28-day testing period are given in Table 3. The results show that all other formulations i.e. HNE-1 to HNE-17 showed liquefaction and phase separation in the third week which showed their instability. HNE-18 was stable for 21 days while HNE-19 was stable for the duration of the preliminary stability study, up to 28 days.

Analysis of long-term stability

Long-term stability analysis results of HNE-18 and HNE-19 are shown in Table 2. The results demonstrated that HNE-18 showed little phase separation, while HNE-19 showed no phase separation for a 90-days testing period. HNE-19 was then selected as a stable formulation for further study.

Thermodynamic experiment

HNE-19 and base-B 19 showed no creaming, cracking, or phase separation after passing centrifugation and six heating/cooling cycles. These results are similar to those observed in a study conducted by Hamed *et al*.[12] These results show the thermodynamic stability of both the nanoemulsions.

Characterization of HNE-19 and B-19

The physical changes i.e., colour, odour, liquefaction or phase separation in HNE-19 and its base-19 were studied for three months (90 days). The results for physical changes in HNE-19 and its base-19 at 8°C, 25°C, 40°C and 40°C with 75% RH are given in Table 4. The formulated HNE-19 and its base-19 were clear and milky white with a pleasant odour. This colour and odor remain same throughout the study period. No phase separation or liquefaction was observed for 90 days which showed both the nanoemulsions were physically stable till 90 days.

pH of B-19 and HNE-19 at four different storage conditions (4°C, 25°C, 40°C, and 40°C with 75% RH) at the different time intervals during the 90-day study period is shown in Table 5. The pH of fresh HNE-19 was 6.74 and fresh B-19 was 6.32. pH starts changing after 45 days. This change was more prominent at 40°C and 40°C+75% RH. At these temperatures, the pH of HNE-19 was 6.02 and 5.95 while the pH of B-19 was 5.91 and 5.99, respectively. After 90 days, the pH of HNE-19 ranged between 5.93–5.83 and B-19 pH ranged between 6.01–5.73 at four different storage conditions.

The average droplet size, zeta potential, mobility, polydispersity, and electrical conductivity of HNE-19 at various time intervals at 25°C are given in Table 6. The droplet size of fresh HNE-19 was 91.07 nm. With the passage of time, an increase in droplet size was observed. The droplet size of HNE-19 after 90 days was 91.07 nm. Zeta potential of fresh HNE-19 was −38.5 mV, and 32.5 mV after 90 days. These results showed a decrease in zeta potential with the passage of time. Polydispersity index of fresh HNE-19 was 0.182, and after 90 days polydispersity index was 0.277 which showed an increase in PDI with an increase in droplet size. The electrical conductivity of fresh HNE-19 was 0.0241. Electrical conductivity values slightly increased with the passage of time. More change in electrical conductivity was observed after 90 days.

Freshly prepared HNE-19 and B-19 were milky white. No turbidity was observed in HNE-19 and B-19 during the 90-day study period which showed the stability of both the nanoemulsions.

The rheological parameters of fresh HNE-19 and B-19 are given in Table 7. Rheograms of honey containing topical nanoemulsion and base have been established in Figs. 1 and 2. The results showed that, as the shear stress and shear rate increased, the viscosity of the HNE-19 and B-19 decreased. This is the property of topical formulations/creams that when the shear stress is applied and increased, their viscosity decreases.

Discussion

Nanoemulsions, because of their enhanced stability, lower viscosity, low surfactant quantity, and fine buildout, are appealing delivery systems.[13] The small droplet size of nanoemulsions provides large surface area and close contact with stratum corneum which make them excellent carriers for drugs, allowing the active moiety to easily reach the site of action. In order to attain the smallest globule size without an ultrasonicator, a modified method was adopted to formulate a nanoemulsion containing honey, because smaller droplet sizes enhance the emulsion stability. As ternary phase diagrams can describe all the possible ratios of mixing components and find probable domains of nanoemulsion, the best way to study all types of nanoemulsion-based formulations is through constructing pseudo ternary phase diagrams by mixing oil, surfactant, and water. A pseudoternary phase diagram helps study all kinds of formulations for the nanoemulsion of water, S_{mix} (Span 80 + Tween 80), and liquid paraffin oil with distinct HLB values of 9, 10, 11, and 12. While formulating nanoemulsions, individual surfactants were not sufficient to produce a film at the interface, which is why S_{mix} was preferred. Formulation HNE-19, having an HLB value of 10, was found to be the most stable because of the availability of an optimal concentration of surfactant mix to form an interfacial film around the oil globules dispersed in the dispersion medium. A stable nanoemulsion was formed using this ratio (T_{80} = 4.26%: S_{80} = 3.74%) of S_{mix}.

Results of the thermodynamic study and centrifugation showed that HNE-19 and base-19 were stable, as no phase separation was seen. Centrifugation was done to ensure that whether the nanoemulsion was stable or not, which implicates the use of centripetal force to split two liquids that are immiscible with each other.[14]

The capability of nanoemulsions to show resistance to changes in its physical and chemical properties with time defines the stability of nanoemulsion. Physical characteristics confirm whether the formulation is physically stable or not, and it is based on visual observations. The emulsification at 25°C, which changed the preparation temperature, caused the nanoemulsion's colour to be milky white. The diameter of emulsion droplet decreased from 10.3 µm to 51 nm, and the nanoemulsion became milky white when the preparation temperature increased from 20 to 70°C, proving the formation of nanoemulsion.[15]

No phase separation, liquefaction, or change in milky white colour was seen in HNE-19 and base-19 at intervals of 0 hr, 24, 48, 72, and on days 7, 14, 21, 28, 45, 60, and 90. Due to the enormously low solubility of the liquid paraffin oil in the aqueous phase or continuing phase, the stability against Ostwald repining was excellent.[16] However, in the HNE-19 and base-19 kept at 40 °C and 75% RH, phase separation occurred at day 90. This phase separation might be due to Ostwald repining or the coalescence of small droplets by the diffusion process. The increase in surface free energy caused the production of bigger droplets. No observed liquefaction provides strong evidence of the stability of nanoemul-

Table 4. Physical changes of B-19 and HNE-19

Condition	Parameters	Fresh		7 days		15 day		30 days		45 days		60 days		90 days	
8°C		B-19	HNE-19	B-19	HNE-19	B-19	HNE-19	B-19	HNE-19	B-19	HNE-19	B-19	HNE-19	B-19	HNE-19
	Color	W	W	W	W	W	W	W	W	W	W	W	W	W	W
	Odor	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Liquefaction	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Phase separation	–	–	–	–	–	–	–	–	–	–	–	–	–	–
25°C	Color	W	W	W	W	W	W	W	W	W	W	W	W	W	W
	Odor	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Liquefaction	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Phase separation	–	–	–	–	–	–	–	–	–	–	–	–	–	–
40°C	Color	W	W	W	W	W	W	W	W	W	W	W	W	W	W
	Odor	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Liquefaction	–	–	–	–	–	–	–	–	–	–	–	–	–	+
	Phase separation	–	–	–	–	–	–	–	–	–	–	–	–	–	–
40°C+RH 75	Color	W	W	W	W	W	W	W	W	W	W	W	W	W	W
	Odor	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Liquefaction	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Phase separation	–	–	–	–	–	–	–	–	–	–	–	–	–	–

B-19, base formulation without honey, HNE-19, active formulation of honey. W= White, – = No, + = Little.

Table 5. pH values for B-19 and HNE-19 kept at 4°C, 25°C, 40°C, and 40°C +75% RH

Storage time	4°C		25°C		40°C		40°C + 75% RH	
	B-19	HNE-19	B-19	HNE-19	B-19	HNE-19	B-19	HNE-19
fresh	6.32	6.74	6.32	6.74	6.32	6.74	6.32	6.74
24 h	6.48	6.45	6.45	6.34	6.29	6.25	6.27	6.15
48	6.50	6.43	6.38	6.26	6.32	6.22	6.25	6.12
72	6.52	6.43	6.36	6.24	6.33	6.19	6.26	6.09
7 days	6.36	6.26	6.27	6.22	6.05	6.17	6.21	6.08
14	6.20	6.20	6.21	6.13	6.01	6.11	6.17	6.05
21	6.27	6.08	6.15	6.02	5.98	6.08	6.08	6.03
28	6.23	6.05	6.13	6.01	5.95	6.04	6.02	5.99
45	6.16	6.03	6.05	5.98	5.91	6.02	5.99	5.95
60	6.07	6.01	6.01	5.94	5.85	5.97	5.91	5.89
90	6.01	5.93	5.98	5.87	5.78	5.91	5.73	5.83

B-19, base formulation without honey; HNE-19, active formulation of honey; RH, relative humidity.

Table 6. Average droplet size and zeta potential, polydispersity, mobility and electrical conductivity of HNE-19 after different time intervals at 25°C

Sample Name	Droplet Size (nm)	Zeta Potential (mV)	Polydispersity Index	Mobility (µm.cm/V.s)	Electrical Conductivity (mS/cm)
Fresh HNE-19	91.07	−38.5	0.189	−2.726	0.0141
HNE-19 after 30 days	145.29	−36.1	0.208	−2.911	0.0236
HNE-19 after 60 days	195.47	−33.4	0.286	−2.684	0.0269
HNE-19 after 90 days	197.06	−32.5	0.277	−2.459	0.0289

HNE-19, active formulation of honey.

Table 7. Rheological parameters of freshly prepared HNE-19 and B-19 at 25°C

Model	Rheological parameter	HNE-19	B-19
Power Law	Consistency index (cP)	127.1	112.9
	Flow index	0.21	0.28
	Confidence of fit (%)	99.8	99.6

B-19, base formulation without honey, HNE-19, active formulation of honey.

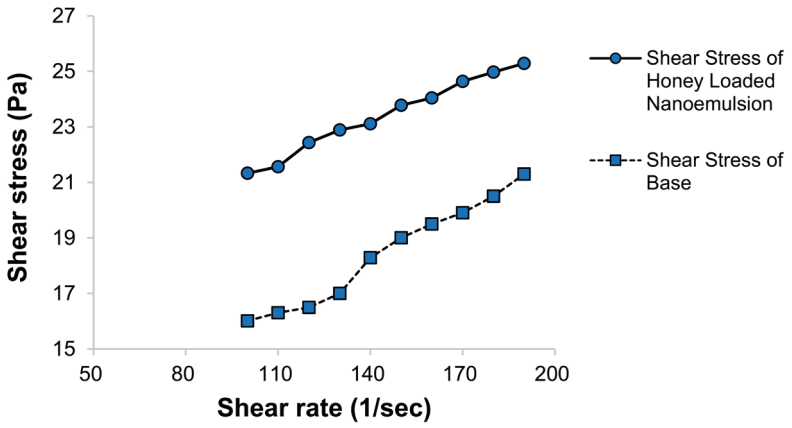

Fig. 1. Rheogram of HNE-19 and B-19 containing honey at 25°C. B-19, base formulation without honey, HNE-19, active formulation of honey.

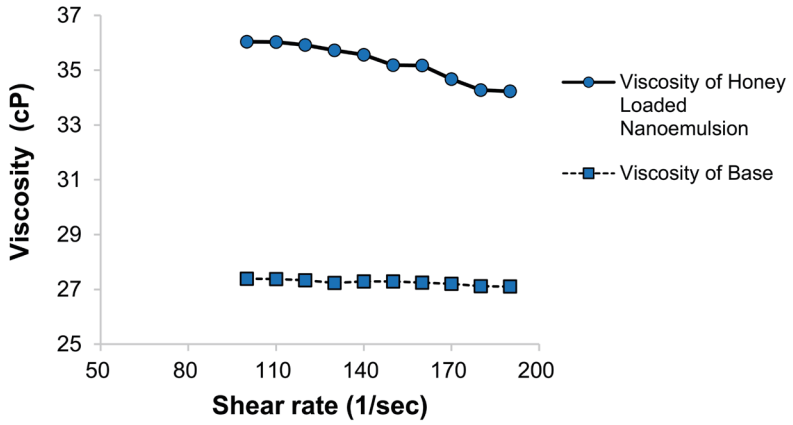

Fig. 2. Viscosities versus shear rate of HNE-19 and B-19 containing honey at 25°C. B-19, base formulation without honey, HNE-19, active formulation of honey.

sions. High-pressure homogenization might be the cause of the absence of liquefaction.

The average droplet size of the freshly prepared nanoemulsion produced by high-energy homogenization was 91.07 nm. The nanoemulsion droplet size at 25°C, after 30 days, 60 days, and 90 days recorded were 145.29, 195.47, and 197.06 respectively. The droplet size of nanoemulsions should be in the range of 20–500 nm.[17] HNE-19 showed no huge increment in the droplet size. However, there is an increase in droplet size due to movements of constituents of honey from higher concentration to lower concentration with time in the nanoemulsion.

The zeta potential is very important in finding the stability of the nanoemulsion; the nanoemulsions are considered the most stable with a zeta potential value of 30 mV. During the 90-day stability study, the zeta potential values of HNE-19 and base-19 were above −30 mV. Fresh HNE-19 had a zeta potential value of -38.5 mV. Subsequent zeta potential values were -36.1 mV, and -33.4 mV after one and two months respectively. The mobility of fresh HNE-19 was -2.31 µm.cm/V.s, after one month it was -2.194 µm.cm/V.s, and after two months it was -2.623 µm.cm/V.s. There was no aggregation or coalescence in the nanoemulsion when the value of the zeta potential was high, i.e., above -30 mV, because the nanodroplets with similar charges repel each other. The type of charge on the surface of the droplet plays a significant role in the transportation of the drug molecules across various physiological barriers.[18]

Electrical conductivity changes may alter the droplet size of nanoemulsions, leading to nanoemulsion instability.[14] In our study, a very small increase in the electrical conductivity of nanoemulsion instability was observed. Hence, by using this parameter, we couldn't convincingly determine the nanoemulsion's stability. Comparable results are likewise reported by Bernardi *et al*.[18] The polydispersity index (PDI) value of the nanoemulsion was below 0.3 during the 90-day testing period, which shows low polydispersity and high fidelity of formulation. In general, this indicates good stability and development methods. A value of PDI close to 1.0 shows a polydisperse system.[19]

Formulations that are stable impart very little change in pH. Normally, the pH of the skin ranges from 4.7 to 5.8.[20] The HNE-19 pH matches the pH of skin. The pH of the fresh samples of HNE-19 and B-19 at the start of the stability study were 6.74 and 6.32, respectively. The pH at 4°C, 25°C, 40°C and 40°C+75%RH at different intervals of the 90-day study period were 5.93, 5.87, 5.91, 5.83, and 6.01, 5.98, 5.78, and 5.73. The minimal change in pH of HNE-19 and B-19 could be due to the stability of the formulation's ingredients. Hence, this shows that during the testing period at different storage conditions, there was no ionization or degradation of chemicals in the formulation.

Turbidity is the haziness, cloudiness, or disturbance of liquid caused by various factors. Turbidity is a key test for determining stability.[21] No turbidity was observed in formulations HNE-19 and B-19 when checked visually at 4°C, 25°C, 40°C and 40°C + 75% RH. The flocculation rate determines how a formulation's turbidity changes over time. Contributions of mixed aggregates, conventional aggregates, and bigger drops resulted in the turbidity of the emulsion.[22]

While formulating a nanoemulsion, rheological parameters are very important. As the shear stress and shear rate increase, the viscosity of the nanoemulsion decreases. HNE-19 showed non-Newtonian flow and shear-thinning pseudoplastic behaviour. This is a property of cosmetics.[21] The consistency index is the measure of apparent viscosity during a shear rate of sec^{-1}. The consistency index of formulation HNE-19 and B-19 was 127.1 cP and 112.9 cP, respectively. The consistency index indicates the viscous nature of the formulation.[23] The rheological analysis of the nanoemulsion disclosed excellent confidence of fit. The confidence of fit for HNE-19 was 99.8, and that for B-19 was 99.6, which supports the fact that with the best-fit curves drawn in accordance with the power law, the nanoemulsion demonstrated striking similarities.[24–26]

Limitations of the study

Limitations of the study were the inability to assess the release constituents and permeation capacity of formulation.

Future directions

The stable nanoemulsion of honey could be evaluated *in vivo* comparatively with conventional topical applications against skin-related diseases like wounds, wrinkles, aging, and inflammations in mammals. Furthermore, it should be emphasized to extend *in vitro* and *in vivo* studies on the release of honey from nanoemulsion and cell-mediated immunity studies. However, variations exist in honey compositions due to their variations in origin and bee types,

which will lead to variable efficacy in terms of prospects and with respect to other novel delivery systems i.e., lipid-based delivery, nanogels, nanoparticles, microneedles etc.

Conclusions

A honey-loaded nanoemulsion (HNE-19) was successfully developed and characterized for stability. The nanoemulsion was thermodynamically stable. With the good rheology and stability of honey, the size of the nanodroplets was below 200 nm. Throughout the 90-day testing period, the nanoemulsion maintained normal pH values that corresponded to skin pH. The emulsion also showed non-Newtonian flow and pseudo-plastic behaviour, which are required for ideal topical formulation. In conclusion, stability studies and characterization showed that nanoemulsions containing honey are exceptional topical delivery formulations.

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Conflict of interest

The authors do not have any conflict of interests to declare.

Author contributions

MA contributed to conceptualization, project administration, methodology investigation, validation and writing- original draft. AA contributed to formal analysis, resources, supervision, validation, visualization, writing, review & editing. HK contributed to data analysis, writing, review & editing.

Data sharing statement

No additional data are available.

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