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L VENGROFF¹, RL HAGAN¹, T HAMOUDA¹, and JR BAKER, JR^{1,2}
¹NanoBio Corporation, ²University of Michigan, Ann Arbor, MI USA

For additional information contact:
John Coffey, Jr.
Phone: (734) 302-9107
E-mail: john.coffey@nanobio.com

ABSTRACT

Purpose: To investigate the long-term physicochemical stability of NB-001, an anti-infective oil-in-water nanoemulsion formulation used for the treatment of herpes labialis.

Methods: Using validated analytical methods, three strengths (0.1% w/v, 0.25% w/v and 0.5% w/v) of the NB-001 formulation were tested over a period of up to 36 months, at appropriate ICH storage conditions, to determine changes in potency, physical appearance, particle size distribution, and pH. High-performance liquid chromatography (HPLC) was used to measure potency. Laser light diffraction-based techniques were used to measure particle size and particle size distribution. Emulsion physical stability was assessed by monitoring changes in physical appearance (i.e. settling, creaming, color change, and phase separation). To assess long term stability each strength was stored in glass vials at 25°C/60%RH and 5°C for up to 36 months. Samples were analyzed at 0, 1, 3, 6, 9, 12, 18, 24 and 36 month intervals. Each strength was placed under stressed conditions (40°C/75%RH) for 6 months and analyzed at 1, 3 and 6-month time points. Given that the samples were stable at 40°C/75%RH, it was not necessary to test samples stored at the accelerated condition of 30°C/65%RH.

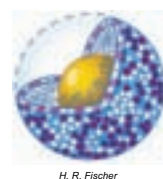
Results: Physical and chemical stability was demonstrated for the three different strengths of NB-001 formulation. Particle size and particle size distribution met pre-set stability specifications, with a mean particle size of approximately 180 nm. There was no evidence of emulsion instability observed at any time point, including under stressed conditions. Potency values showed little change from the 0.1% w/v and 0.5% w/v target initial values for NB-001.

Conclusions: Stability data support a shelf life of up to three years for NB-001. High energy emulsification in conjunction with a unique composition of ingredients results in a pharmaceutically stable oil-in-water nanoemulsion formulation. Pre-clinical data indicate that this topical nanoemulsion formulation is able to penetrate through hair follicles and skin pores to reach the site of infection, while minimizing skin irritation. Clinical data from Phase 2 clinical trials in herpes labialis with the NB-001 formulation demonstrate this formulation is efficacious in the treatment of herpes labialis.

BACKGROUND

- NB-001 is a novel antiviral nanoemulsion with demonstrated efficacy in Phase 2 clinical trials for the treatment of herpes labialis.
- Reduction of emulsion particle size is not the leading determinant of emulsion stability. Formulation components, and achieving the optimal ratio of these components, also play a key role in maintaining droplet size, thereby increasing emulsion shelf life.
- For NB-001, high energy emulsification, in conjunction with a unique composition of ingredients results in a pharmaceutically stable oil-in-water nanoemulsion

Figure 1
Nanoemulsion
droplet (NB-002).



- Cetylpyridinium Chloride
- Polysorbate
- Oil

H. R. Fischer

RESULTS

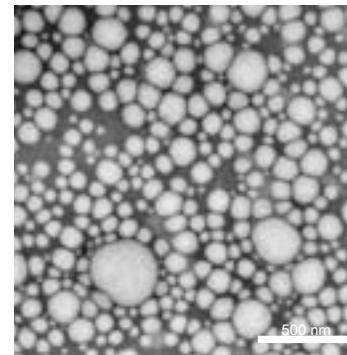


Figure 2. Electron micrograph of NB-001, Time = initial (34,000X)

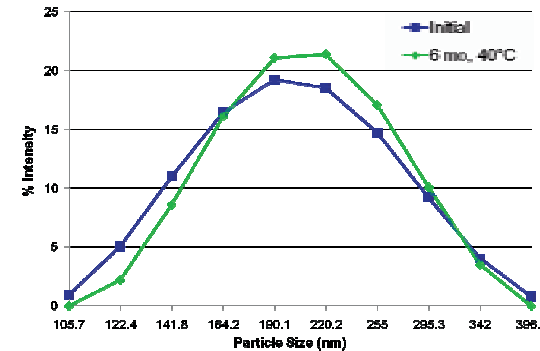


Figure 3. Particle size distribution of NB-001 0.3% (w/v) over six months at 40 °C

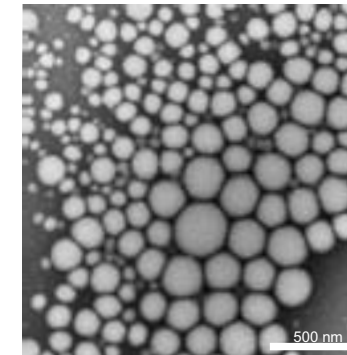


Figure 4. Electron micrograph of NB-001, Time = 6 mos, 40 °C (34,000X)

- No change was noted in appearance of the emulsion, by either visual inspection (data not shown) or electron microscopy (Figures 2 & 4) throughout the course of the study.
- There was no change in the mean particle size distribution (Figure 3) or mean particle size (Figure 5) over time.
- There was no change in the potency measurements (Figure 6) or pH (Figure 7) over time.

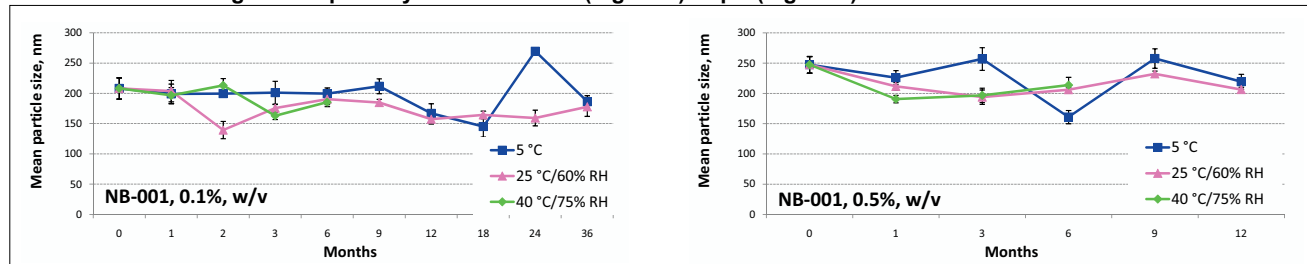


Figure 5. Mean particle size results over time for the bracketing strengths of 0.1% and 0.5%, w/v

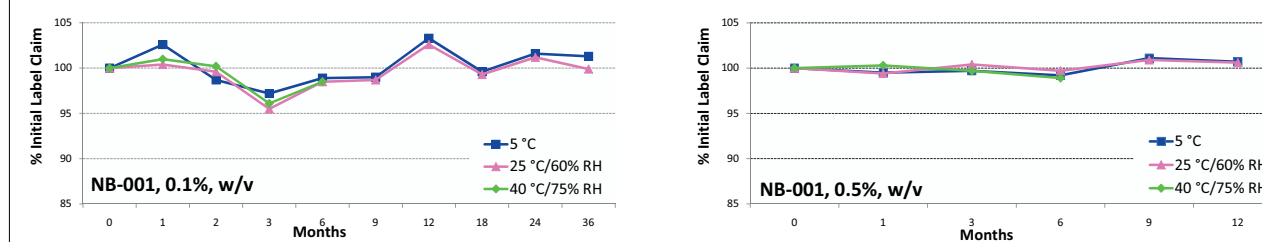


Figure 6. Cetylpyridinium chloride (CPC) potency for the bracketing strengths of 0.1% and 0.5%, w/v.

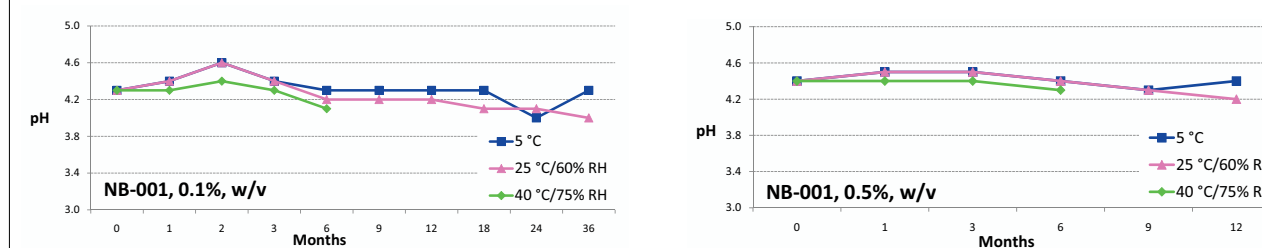


Figure 7. pH measurements over time for the bracketing strengths of 0.1% and 0.5%, w/v

METHODS

- NB-001 is an oil-in-water emulsion with a mean droplet diameter of ~180 nm, prepared with pharmaceutical grade excipients using a proprietary manufacturing method.
- Samples were stored in glass vials at 25°C/60%RH and 5°C for up to 36 months. Samples were analyzed at 0, 1, 3, 6, 9, 12, 18, 24 and 36 month intervals. Each strength was placed under stressed conditions (40°C/75%RH) for 6 months and analyzed at 1, 3 and 6-month time points. Given that the samples were stable at 40°C/75%RH, testing at the accelerated condition of 30°C/65%RH was not necessary.
- Appearance was visually assessed for evidence of creaming, settling, separation and color change.
- Particle size evaluation was conducted by laser diffraction light scattering using a Beckman Coulter N4 Plus Particle Size Analyzer. Samples are appropriately diluted with deionized water prior to measurement.
- Potency was evaluated by measurement of CPC, the marker for the nanoemulsion, using a validated HPLC method (Figure 8).
- pH was determined using a standard calibrated pH meter.
- Transmission Electron Microscopy: NB-001 was placed on 200 mesh carbon coated copper grids for 10 mins. Grids were blotted, air-dried, and rinsed twice with double distilled water. Grids were stained using 1.0 % uranyl acetate and examined using a Philips CM-100 transmission electron microscope at 100 kV.

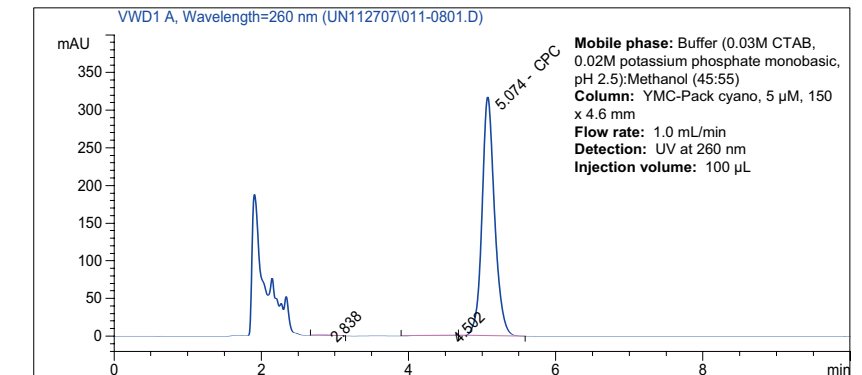


Figure 8. NB-001 chromatogram and conditions

CONCLUSIONS

- NB-001 is a novel antiviral nanoemulsion with a shelf life of up to three years.
- High energy emulsification, in conjunction with a unique composition of ingredients, results in a pharmaceutically stable oil-in-water nanoemulsion.
- This topical nanoemulsion formulation is able to penetrate through hair follicles and skin pores to reach the site of infection, while minimizing skin irritation (Ciotti, et al. 2008 AAPS. T3031).
- Clinical data from Phase 2 clinical trials in herpes labialis with the NB-001 formulation demonstrate this formulation is efficacious in the treatment of herpes labialis (Jones, et al. 48th Annual ICAAC. V-3771)