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# Development of a w/o/w-Emulsion Containing *N*-Acetylcysteine for Cosmetic Use

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## Abstract

*N*-Acetylcysteine was known to be used as a mucolytic agent having significant antioxidant activity. It has been reported to be used to alleviate or improve various cosmetic conditions and dermatological disorders including changes or damage to skin associated with intrinsic or extrinsic aging recently. However many benefits regarding to its antioxidant effects were reported, its probable potential moisturizing effects have not been stated yet. Since *N*-acetylcysteine has similar effects as vitamin C, it might be assumed that it could also increase skin hydration by its antioxidant capacity. The aim of this work was to investigate the feasibility of a topical delivery system as a multiple emulsion containing *N*-acteylcysteine and compare the moisturizing potential of this formulation with a base multiple emulsion and vitamin E which was chosen as a popular moisturizer.

## Keywords

*N*-Acetylcysteine • Multiple emulsion • Moisturization • Vitamin E • Topical delivery

## Introduction

*N*-Acetylcysteine (NAC), besides being a mucolytic agent is a substance having significant antioxidant activity when used in combination with β-caroten, Vitamin A and other retinoids [1, 2]. In recent years NAC has been used as an additive in treatment of wounds and burns and as anti-wrinkle agent [3–5]. It is also used in topical treatment of neonatal

ichthyosis [6–8]. Protective effect of NAC in irritant and contact hypersensitivity reactions was recorded [9]. Interactions between NAC and ascorbic acid in modulating carcinogenesis was also reported [10]. There is no study recorded regarding to its application in various cosmetic preparations In this study, it was attempted to prepare w/o/w emulsions of NAC for cosmetic use and investigated in respect of stability and NAC moisturizing efficacy.

Multiple w/o/w emulsions are vesicular systems in which very small water droplets are entrapped within oil drops [11] themselves dispersed in an aqueous phase [12]. They have many potential applications in various fields such as pharmaceutics and cosmetics, as they might protect encapsulated substances and enable incompatible substances to be incorporated in the same formulation; moreover, prolonged action after administration can be expected [13].

In our study, we have entrapped NAC and Vitamin E (vit. E) in both different and same w/o/w multiple emulsion formulations: NAC being in the inner aqueous phase and vit. E in the oil phase and also base multiple emulsions were prepared with no active ingredient and their moisturizing effects were compared with each other on the inner forearms of ten healthy female volunteers.

## Results and discussion

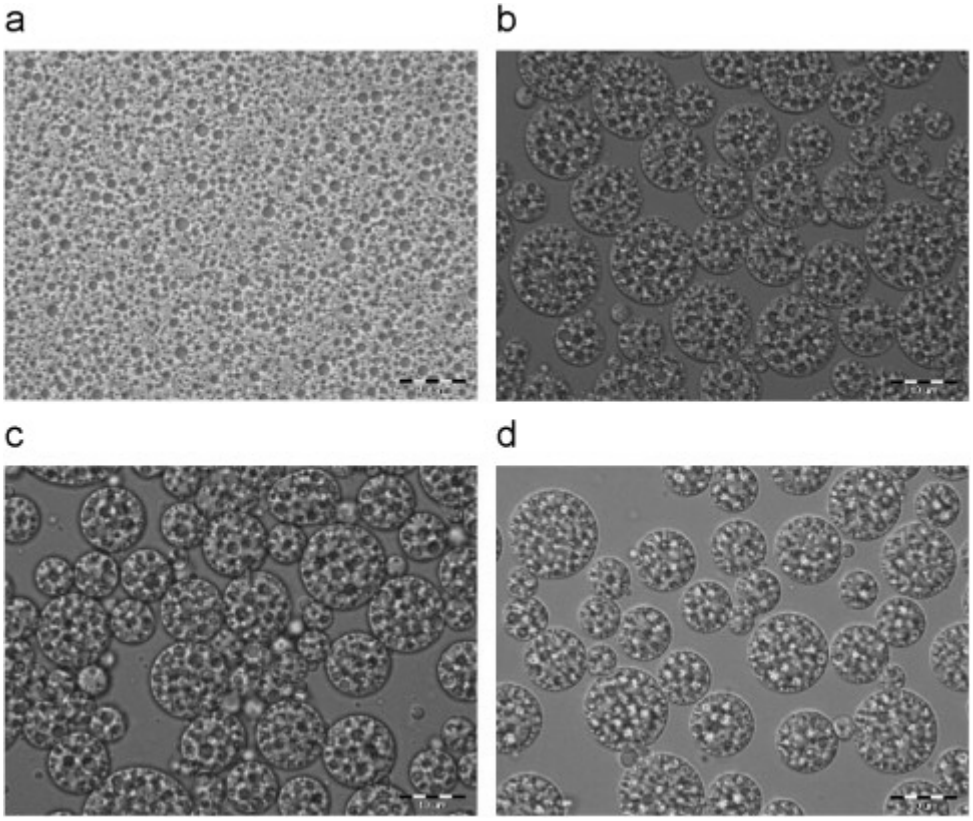


**Fig. 1.** w/o, w/o/w emulsions (F2*) just after preparation (a, b) and at the end of stability tests for F2* (c) and F3* (d)

### *Characterization of w/o/w emulsions*

Photomicrographs of primary and multiple emulsions immediately after preparation and at the end of stability tests are shown in Fig. 1.

### *Effects of production parameters on the stability of multiple emulsions*

The composition and production parameters of the formulations investigated in this study are shown in Tables 1 and 2.

The first step of the study was to optimize the production parameters. Datas obtained in case of optimized conditions for primary emulsion formulation is as follows: optimal oil phase volume was found as 20 %, surfactant was chosen among the ones tested namely Span 20, Span 80, Span 20–80 (1:1) and ABIL EM 90. We have conducted the studies with all of these and since ABIL EM 90 gave the most satisfactory results, it was chosen and used in our study, mixing time was chosen as 15 min. and mixing rate as 1500 rpm and additional 15 min. with 800 rpm respectively. Temperature was maintained as 80ºC. Magnesium sulphate was added as an electrolyte to the inner water phase.

Datas obtained in case of optimized conditions for emulsion formulation is as follows: optimal primary emulsion phase volume was chosen as 80 %. Mixing time and rate were 45 min. 500 rpm for the second step emulsification.Hydrophilic surface active agent was chosen to be Tween 80 among the others tested. Addition of the primary emulsion to the outer water phase was found to be slowly by small portions. Optimal preparation condition for temperature was found as 25ºC. In order to increase the density of the outer phase of multiple emulsion, 2 % Carbopol 934 was used. As mixing shaft, triple propeller mixer was chosen.

Organoleptic and microscopic investigations, particle size analysis, centrifuge tests, rheological analysis were carried out and stability tests were performed at various temperatures on all formulations as usually done to assess stability of multiple emulsions [14].

**Tab. 3.** Results of microscopic analysis

| **Formulations** | **Days** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4 ± 1°C | | | | 25 ± 1°C | | | | 40 ± 1°C | | | |
| | 7 | 30 | 60 | 90 | 7 | 30 | 60 | 90 | 7 | 30 | 60 | 90 |
| F-1* | S | S | S+ | S+ | S | S | S | S+ | S | S | S+ | + |
| F-2* | S | S | S | S | S | S | S | S | S | S | S | S |
| F-3* | S | S | S | S | S | S | S+ | S+ | S+ | S+ | ++ | ++ |
| F-4* | S | S | S | S+ | S | S | S | S+ | S | S | S+ | S+ |

S: Stable form; +: Decrease in viscosity; ++: Beginning of phase separation; +++: Partial phase separation; ++++: Complete phase separation (n=3)

As can be seen from Table 3, results of microscopic analysis after storing at 4°C, 25°C and 40°C, formulations F1*, F2* and F4* showed no signs of phase separation after 3 months. Only F3* showed beginning of phase separation when stored in 40ºC after 60 days. In F1* in 40ºC after 90 days, a decrease in viscosity was observed. This situation

may be attributed to viscosity increasing effect of NAC in case of F2* and F4* which were found more stable when compared to F1* and F3*.

In Table 4, data of globule size analysis is shown. In all formulations tested, globule sizes weren't grown bigger; instead smaller sizes were observed after 3 months at all test conditions. This situation shows that since globule size doesn't become bigger they don't have a tendency to agglomerate which leads to phase separation. Results of Table 3 also confirm these results. Globule sizes don't increase and the formulations don't have phase separation. When the data is analyzed in Table 4, it can easily be seen that at 4°C and 25°C, decrease in globule sizes are not significant thus decrease was found more at 40°C which is an accelerated condition.

Viscosity values which are also important for stability criteria are shown in Table 5. When the results are analysed, it could be concluded that there was gradual decrease in viscosity in all formulations tested when time passes. Viscosity of F1* was 24657 m.Pa.s at the production date and was found 21838 m.Pa.s instead of (22492) and 20745 m.Pa.s instead of (21838) m.Pa.s after 60 and 90 days respectively. Viscosity of F4* was 30047 m.Pa.s at the production date and were found found 26711 m.Pa.s and 24578 m.Pa.s after 60 and 90 days respectively at 25ºC. This much decrease in viscosity doesn't effect the stability which is also confirmed by organoleptic controls, microscopic and particle size analyses. Decrease in viscosity was not attributed to microbial contamination since microbial tests were also performed and no contamination was detected. Organolaptic controls also revealed no change in colour over the storage time.

**Tab. 4.** Data of globule size (μm) analysis by Malvern Mastersizer

| **Formu-lations** | | **Mean diameter [μm]** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PD | 4°C | | | 25°C | | | 40°C | | |
| | | | 30 days | 60 days | 90 days | 30 days | 60 days | 90 days | 30 days | 60 days | 90 days |
| F1 | LD50 | 8.75 | 8.62 | 8.31 | 8.19 | 7.96 | 7.84 | 7.52 | 7.24 | 6.38 | 5.44 |
| | LD95 | 14.46 | 14.25 | 14.22 | 14.24 | 14.31 | 14.28 | 14.21 | 11.12 | 12.05 | 10.76 |
| | LD99 | 30.16 | 30.42 | 30.48 | 30.42 | 29.88 | 29.92 | 29.36 | 26.39 | 25.21 | 24.09 |
| F2 | LD50 | 8.26 | 8.31 | 7.06 | 6.39 | 8.02 | 7.63 | 6.49 | 6.89 | 6.20 | 5.84 |
| | LD95 | 15.21 | 16.05 | 16.69 | 16.38 | 15.49 | 15.48 | 15.07 | 12.55 | 11.43 | 10.18 |
| | LD99 | 34.48 | 34.66 | 34.71 | 34.11 | 29.88 | 29.92 | 29.36 | 28.41 | 27.97 | 25.41 |
| F3 | LD50 | 9.07 | 8.05 | 7.61 | 7.09 | 6.24 | 6.01 | 5.81 | 5.12 | 5.01 | – |
| | LD95 | 21.96 | 20.58 | 20.50 | 19.31 | 20.94 | 19.15 | 20.39 | 20.42 | 15.05 | – |
| | LD99 | 40.15 | 41.26 | 41.08 | 41.39 | 40.36 | 39.36 | 42.55 | 39.21 | 24.6 | – |
| F4 | LD50 | 10.61 | 9.52 | 9.22 | 8.23 | 8.11 | 7.79 | 6.57 | 7.15 | 6.03 | 5.23 |
| | LD95 | 35.15 | 34.46 | 34.93 | 36.74 | 35.23 | 32.67 | 32.71 | 31.38 | 26.43 | 24.18 |
| | LD99 | 64.88 | 65.99 | 64.35 | 65.48 | 65.04 | 65.18 | 64.49 | 53.12 | 51.36 | 49.23 |

LD50, LD95, LD99: Laser diffraction (LD) data of formulations. LD50 means that 50 % of the globules are below the given size; PD: Production date (n=3)

**Tab. 5.** Viscosity values of the formulations at 25°C

| Formu-lations | Days | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | PD | | 7 | | 30 | | 60 | | 90 | |
| | Viscosity (m.Pa.s) | SD (±) | Viscosity (m.Pa.s) | SD (±) | Viscosity (m.Pa.s) | SD (±) | Viscosity (m.Pa.s) | SD (±) | Viscosity (m.P.a.s) | SD (±) |
| F1* | 24657 | 965 | 23516 | 709 | 22492 | 687 | 21838 | 551 | 20745 | 425 |
| F2* | 28504 | 882 | 27124 | 767 | 26475 | 559 | 25326 | 697 | 22561 | 585 |
| F3* | 26235 | 721 | 25031 | 631 | 23125 | 584 | 20063 | 483 | – | – |
| F4* | 30047 | 984 | 29216 | 768 | 27996 | 824 | 26711 | 634 | 24578 | 570 |

PD: Production date (n=3)

The current investigations have shown that the choice of preparation conditions of formulations such as oil phase volume, temperature, mixing rate and time, addition of electrolytes, type of mixing shaft and densities of internal and external phases play an important role on the stability of multiple emulsions which is of prime importance. After long investigations, optimal conditions were chosen and stable multiple emulsions could be produced with all ingredients involved in this study. On the second part of the study, after obtaining the stable multiple emulsions, panel tests were performed and depending on the results obtained from Corneometer measurements, formulation F4* containing NAC (5%) and vit.E (5%) which was prepared by polymeric lypophilic surfactant (Abil EM90) was found as the most suitable w/o/w formulation with respect to stability and effectiveness followed by F2*, F3* and F1* respectively (Fig.2). Hence no significant difference was observed statistically among F2* and F3* (ns) when compared. As can be seen from Table 6, data obtained showed that w/o/w emulsion containing NAC alone (F2*) moisturized skin not less than the one containing vit.E (F3*) which has been known and used as a popular moisturizer for a long time [15].

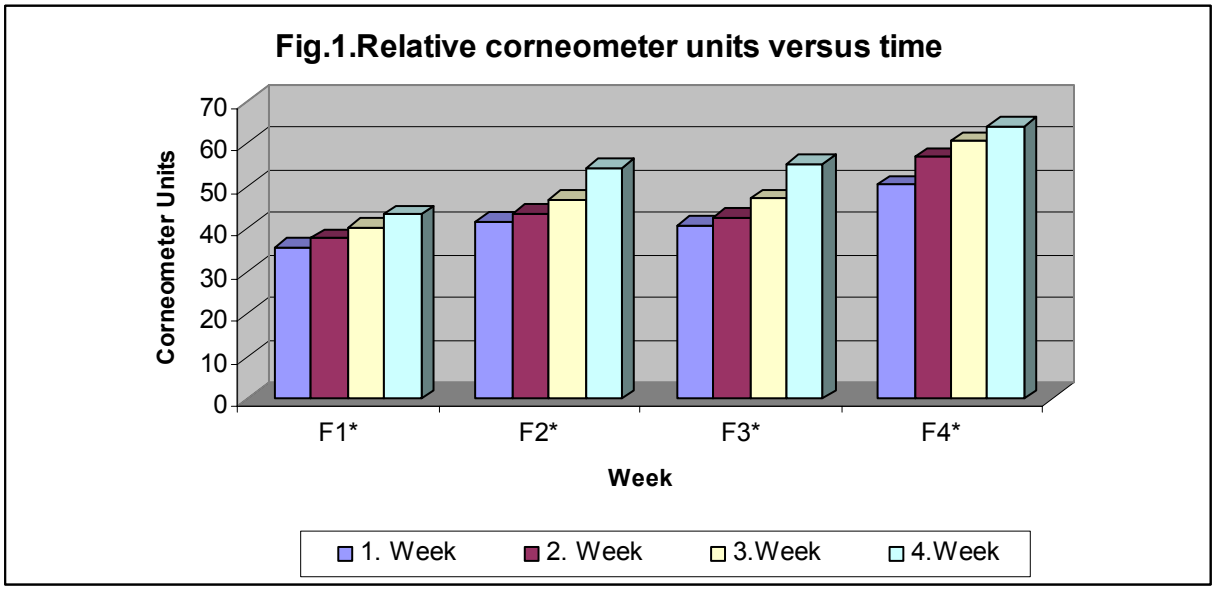


**Fig. 2.** Relative corneometer units versus time (week)

**Tab. 6.** Evaluation of statistical data of the formulations as q and p- values compared to each other in moisturization tests.

| Formulation | Q | p-value |
|---|---|---|
| F1* vs F2* | 6.483 | p<0,001 |
| F1*vs F3* | 7.549 | p<0,001 |
| F1*vs F4* | 12.768 | p<0,001 |
| F2* F3* | 1.610 | Ns |
| F2* F4* | 4.025 | p<0,01 |
| F3* F4* | 4.392 | p<0,05 |

## Experimental

### *Chemicals*

NAC was supplied by Bilim Pharmaceutics, Turkey. Vitamin E was obtained form Fluka Chemika, Switzerland. Abil was donated by Goldschmidt, Germany. Cellulose acetate membrane was from Sartorius, Germany. All other substances were of analytical grade having chemical purity.

Microscope (SOIF H-ZSX/Syne Master 510S), Viscosimeter (Brookfield DVII), Mechanical Mixer (KİKA labortechnik RW20 DZM), Malvern (Mastersizer 2000)

### *Preparation of w/o/w emulsions*

Multiple emulsions are complex, inherently unstable systems which are unlikely to be commercially acceptable as drug delivery systems until problems of their instability in vitro and in vivo are solved [16]. It has been reported that to improve photostability, attempts were made to polymerize appropriate monomers in the aqueous phases of w/o/w multiple emulsions. The aim was to reduce the potential for coalescence of the internal aqueous droplets. Because the preparation of a w/o/w system is a two-step procedure, it is possible to modify either the primary aqueous phase which subsequently becomes the continuous aqueous phase. Each aqueous phase can be gelled by insitu polymerization.

The aim of our research was to prepare stable w/o/w multiple emulsions and ensure that their physiochemical properties were not modified by adding the chosen cosmetic actives (*N*-acetylcysteine and vitamin E). We obtained multiple emulsions using a two-step process. Abil EM 90 was used in the lipophilic phase and $MgSO_4 \cdot 7H_2O$ in the internal aqueous phase of primary emulsion as in a similar study [17].

**Tab. 1.** Formulations of primary emulsions

| Formulations | Ingredients % | | | | | |
|---|---|---|---|---|---|---|
| | Oil Phase (Liquid parafine) | Abil EM 90 | $MgSO_4 \cdot 7H_20$ | Water | NAC | vit.E |
| F1 | 20 | 4 | 0,7 | 75,3 | – | – |
| F2 | 20 | 4 | 0,7 | 70,3 | 5 | – |
| F3 | 20 | 4 | 0,7 | 70,3 | – | 5 |
| F4 | 20 | 4 | 0,7 | 65,3 | 5 | 5 |

Ratio of ingredients in multiple emulsion is as 80:4: 16 (Primary emulsion:Tween 80:Carbopol 2%)

Multiple emulsions were prepared by using lypophilic surfactant ABİL EM-90 and hydrophilic surfactant Tween 80 by a two step emulsification method. In order to improve the stability of multiple emulsions, preparation conditions of formulations were optimized by changing factors such as oil phase volume, temperature, mixing rate and time, addition of electrolytes, type of mixing shaft and densities of internal and external phases. NAC (5 %) was added to internal water phase and vit. E (5 %) was added to oil phase of the multiple emulsions.

**Tab. 2.** Constituents of w/o/w emulsions (%)

| Formulatıon | primary emulsion | water | tween 80 |
|---|---|---|---|
| F1* | F1 | 16 | 4 |
| F2* | F2 | 16 | 4 |
| F3* | F3 | 16 | 4 |
| F4* | F4 | 16 | 4 |

### *Evaluation of the emulsions*

Primary and multiple emulsions were analyzed to assure the formation and stability of the emulsion systems.

### *Physical analysis*

Primary and multiple emulsions were analyzed organoleptically (color, thickness, look, feel) and physically (creaming and phase separation).

### *Types of emulsions*

Types of emulsions were determined by diluting the emulsion with water and oil separately.

### *Microscopic test*

Multiple emulsions were analyzed under microscope (SOIF H-ZSX/Syne Master 510S) with 100X magnification, using immersion oil [18].

### *Globule size analysis*

Globule sizes of multiple emulsions were determined (Malvern, Mastersizer 2000) for the freshly prepared emulsions and for the emulsions kept at various conditions as 4°C, 25°C and 40°C. Analyses were performed on the 1st week and1st, 2nd and 3rd month.

### *Viscosity measurements*

Viscosity of the formulations were tested at room temperature using a Brookfield DV II viscometer (Germany) equipped with TF Helipath spindle at the first day after production date. Measurements were made at 100 rpm in three replicates and viscosity values were recorded as recorded as m.Pa.s.

### *Skin moisturization measurements*

All formulations containing NAC, vit. E, NAC-vit. E combination and **placebo** formulations were applied in standardized fashion (temperature 22°C, relative humidity 60 % [19, 20] on the inner arms of ten healthy female volunteers (23–35 years) twice daily for 4 weeks. All volunteers provided written informed consent for participation. Baseline values (prior to first application of formulations), stratum corneum hydration and the moisturizing effects of the substances on each test area were determined by a Corneometer CM 820 (Courage & Khazaka, Germany). Measuring method of the Corneometer is based on the physical pirinciple of a common capacitor, a complex of two metal plates electrically insulated by a medium that acts as a dielectric. An excess of electrons is built up on one plate (negative charge) and an electron deficiency (positive charge) on the other plate. The quantity of this stored electric charge is called the capacity. The measuring capacitor shows changes of capacitance according to the moisture content of the skin. The probe is connected to a computer, which processes and displays the current reading [21–23]. Corneometer has been widely used to assess skin hydration and moisturizing effects of substances on skin [24–27].

### *Data treatment and statistics*

Statistical evaluation of the formulations for skin moisturization studies were performed by Student's t test (GraphPad InStat).

## Conclusions

Results of this study showed that w/o/w emulsion containing 5% NAC and 5% vit.E gave the best results in respect of stability and moisturizing effect. It has been known that vit. E could be used as an effective moisturizer and for this reason vit. E was incorporated in the formulations of our study for comparison when assesing the moisturizing effect of NAC. Since there was no significant difference in case of moisturization between our formulations containing NAC (F2*) or vit. E. (F3*). It was assumed that NAC could be used as a moisturizer with a similar effect like vit. E. Hydrated stratum corneum provides a flexible, intact barrier, making it highly protective, this effect is desired and it has been shown for the first time in our study that it could be gained by using NAC [28, 29]. Therefore it could be concluded that progress was achieved in trial to introduce a new active ingredient for cosmetic industry.

## Authors' Statement

### *Competing Interests*

The authors declare no conflict of interest.

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