Modern and safe antimicrobial stabilization of cosmetic products

KARIN M. STOFFELS
Schülke & Mayr GmbH Robert-Koch-Strasse 2 Norderstedt, 22851, Germany

ABSTRACT: The microbial safety of cosmetic products is an important consideration. It is integral to ensuring the stability of the products for the entire shelf-life and to preventing damage to the health of the consumers. Currently, the number of accepted substances for microbial stabilization is limited due to legal restrictions or public discussion. Hurdle technology and the intelligent combination of multifunctional additives help to design microbiologically stable products. The synergistic blend of phenethyl alcohol and ethylhexylglycerin is an effective stabilization system, while consisting of 90 percent nature-identical material.

INTRODUCTION

The regulatory framework for preservatives varies in different regions. According to the EU Cosmetics Directive 76/768/EEC, preservatives are substances “for the primary purpose of inhibiting the development of microorganisms” in cosmetic products. These substances are listed in Annex VI of the EU Cosmetics Directive (1). Formulated preservatives in Annex II of the Japanese “Standard of Cosmetics” (2). Under the Federal Food, Drug, and Cosmetic Act in the USA, cosmetic ingredients, with the exception of colour additives, “do not require FDA approval before they go on the market. Companies who market cosmetics have the legal responsibility to ensure the safety of their products” [3].

In addition to legal requirements, the options for microbial stabilization are restricted by marketing demands. The movement to preservative-free or natural products and restrictions on certain actives, i.e. formaldehyde-releasers, isothiazolinones, etc., are increasing.

The desire to avoid cosmetic preservatives extends to all categories of traditional preservatives, leading to a limited number of accepted actives. Parabens, for example, are some of the most commonly used preservatives due to their excellent efficacy and low sensitizing potential (4, 5). Public discussion has caused some manufacturers to avoid using these materials in new formulations.

The claim “paraben-free” on personal care products first appeared 2005. According to market research company Mintel, in 2011 this claim was used for 9 percent of total beauty and personal care launches worldwide; mainly in North America, Europe, and Japan. In contrast, the “preservative-free” claim is still of minor importance. Less than one percent of new cosmetic products were launched worldwide with this claim; mainly in Japan (3 percent of total launches) (6). It is debatable whether these claims meet the consumer needs or increase their uncertainty of the safety of cosmetic products.

In addition, the trend to natural and sustainable products is on-going. According to Kline & Company, the global natural personal care segment in 2010 is about USD 23 bn. This is a small segment in the personal care market (~USD 300 bn). However, the sales growth of 15 percent from 2009 to 2010 and the expected annual sales growth of 12 percent through 2015 are remarkable (7, 8).

There is still no universal definition of “natural” within the personal care market. Looking into the ingredients of natural products, Kline differs in “truly natural” and “natural-inspired” products and states that 76 percent of global sales of natural products are in the group of “natural-inspired”. The majority of ingredients in “natural-inspired” products are synthetic. These are combined with some natural ingredients. The “truly natural” criteria often include higher proportions of natural ingredients and mainly avoid less desirable ingredients such as silicones, ethoxylates, chemical UV filters, and synthetic preservatives (7). However, products including high percentages of natural components are more susceptible to microbial contamination, either because the raw material contains a high initial bio burden or because most natural materials are an excellent medium for microbial growth. Due to these obstacles, formulators are interested in finding novel ways to keep cosmetic products microbiologically stable.

TECHNOLOGIES IN USE

One formulation technique to achieve self-preserving formulations or to reduce the need for preservatives is known as “hurdle technology”. This technique has been used in the food industry since the 1970’s (9). Hurdle technology describes the intelligent formulation using different preservation factors. Good manufacturing practices, appropriate packaging, careful choice of the form of emulsion, low water activity and low or high pH values can be used to control microbial growth in the absence of traditional preservatives (10).

Additionally, chelating agents can be used to increase the efficacy of antimicrobial substances. Chelating agents remove metal ions from the cell membrane of the microbes forming complexes. The lack of metal ions weakens the cell membrane and allows a better penetration of the preservatives (11). A common chelating agent providing this effect is ethylenediaminetetraacetic acid (EDTA). Due to widespread use...
It exhibits effective inhibitory action on microbes, especially Gram-negative bacteria. The mechanism of action of phenethyl alcohol is at the level of the cell membrane, causing a breakdown of the cellular permeability barriers. This alteration of membranes is assumed to lead to disruption of DNA and protein synthesis (14).

**Ethylhexylglycerin**

Ethylhexylglycerin is a multifunctional cosmetic ingredient with excellent deodorising and skin care properties. It has a limited activity against spoilage-causing bacteria. However, if combined with alcohols, antimicrobial stabilizers like glycols or preservatives, ethylhexylglycerin acts as a booster, enhancing their antimicrobial activity (15).

The chemical structure must be considered to explain the mode of action of this boosting effect. Due to its surfactant-like structure, ethylhexylglycerin has the capability to significantly reduce the surface tension of water. This improves the contact of the antimicrobial actives with the cell membranes resulting in better penetration and therefore a higher efficacy (15).

**SOLUTION FOR ANTIMICROBIAL STABILIZATION**

The combination of ethylhexylglycerin with the fragrance ingredient phenethyl alcohol has been investigated with regards to its antimicrobial properties and to a possible boosting effect of ethylhexylglycerin on phenethyl alcohol.
BIOCIDES AND PRESERVATION

Test material
All described tests have been done with nature-identical phenethyl alcohol. Nature-identical materials, synthetic versions of the same chemistries found in nature, offer a way to harness the best of nature without relying on the availability or quality of the materials that exist in nature.

sensiva® SC 50 was used as single ingredient ethylhexylglycerin. The test combination sensiva® PA 20 is a mixture of both ingredients in the ratio of 9 : 1.

Methods
Germ count reduction test
The purpose of the germ count reduction test is to determine exposure times for antimicrobial substances. Oil-in-water emulsions are inoculated in the laboratory. The titre of the suspension is approx. 10⁸ cfu/ml for bacteria, 10⁶ cfu/ml for yeasts and 10⁷ cfu/ml for moulds. Nutrient media are CSA (tryptone-soya-agar) for bacteria and SA (sabouraud-dextrose-agar) for yeast and mould. The plates are incubated for 48 hours at 37°C for all tested germs except the mould Aspergillus niger which is incubated for 48 hours at 25°C.

Challenge Test (schülke Koko Test)
A mixture of bacteria, yeast and moulds are inoculated 6 times (once a week) into the test material, with the goal of keeping the test material germ free for this period. The inoculum contains pathogenic microorganisms as germs which are well known for product spoilage. All species have to be cultivated separately and mixed directly before the addition, to ensure a constant composition and germ count of the inoculum. Its germ count is approx. 10⁸ cfu/ml, which means a germ count of approx. 10⁶ cfu/ml in the sample. Nutrient media are CSA (tryptone-soya-agar) for bacteria and SA (sabouraud-dextrose-agar) for yeast and moulds.

Results
Figures 3, 4 show germ count reduction tests in oil-in-water emulsion for the bacteria Staphylococcus aureus and Escherichia coli, as well as the mould Aspergillus niger and the yeast Candida albicans. The tests have been conducted with phenethyl alcohol and ethylhexylglycerin individually, and with a mixture in the ratio of 9 : 1 (sensiva® PA 20). Phenethyl alcohol achieved a reduction of the spoilage causing germs, whereas ethylhexylglycerin in this small amount had no effect when used alone. The killing rate of phenethyl alcohol was increased significantly for all germs by pairing it with ethylhexylglycerin at a ratio of 9 : 1.

Test Material

<table>
<thead>
<tr>
<th>Inoculation Cycles</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil-in-water Cream unpreserved</td>
<td>B</td>
<td>M</td>
<td>M</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>+0.9% PEA</td>
<td>B</td>
<td>M</td>
<td>M</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>+0.1% EHG</td>
<td>B</td>
<td>M</td>
<td>M</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>+0.05% PEA (0.75% sensiva® PA 20)</td>
<td>B</td>
<td>M</td>
<td>M</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>+0.05% EHG (1% sensiva® PA 20)</td>
<td>B</td>
<td>M</td>
<td>M</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>+0.0% PEA</td>
<td>B</td>
<td>M</td>
<td>M</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>+0.0% EHG</td>
<td>B</td>
<td>M</td>
<td>M</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>+0.05% EHG</td>
<td>B</td>
<td>M</td>
<td>M</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>+0.05% EHG</td>
<td>B</td>
<td>M</td>
<td>M</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
</tbody>
</table>

Table 1. Formulation of oil-in-water emulsion.

Table 2. Formulation of water-in-oil emulsion.

Table 3. Challenge test (schülke Koko test) with two formulations.
Pure phenethyl alcohol (0.45/0.9 percent) reduced all germs to zero after 168 hours. The synergistic blend achieved the reduction to zero after 48 hours for C. albicans and S. aureus and after 6 hours for E. coli. A faster decrease was even observed in the persistent mould A.

The synergistic effect of the blend has also been shown in challenge tests (schülke Koko test) with two formulations (Table 1-3).

Based on experience, a cosmetic product without growth of microorganisms after six inoculation cycles (six weeks) can be considered microbiologically stable for 30 months, which is recommended for cosmetic products (16).

The combination of phenethyl alcohol with ethylhexylglycerin (sensiva® PA 20) stabilizes the o/w cream well at a level of 1 percent. The w/o cream is well stabilized at a level of 0.75 percent. The single ingredients in the corresponding concentrations show no efficacy in the Koko test (Table 1).

Ethylhexylglycerin was found to be an excellent booster for the antimicrobial capacity of phenethyl alcohol. The killing rate in the germ count reduction tests was accelerated. The boosting effect was confirmed by the results of the challenge tests. Depending on the composition of the formulations, 0.75 and 1 percent of a blend of these two components stabilized the emulsions effectively.

### SUMMARY

The contamination of cosmetic products is a high risk. The concept of hurdle technology is not always sufficient to achieve self-preserving systems or not all necessary factors can be implemented. The intelligent combination of multifunctional ingredients using synergism and boosting effects can be a solution to protect products from microbial contamination, are friendly to the skin and are accepted by the consumers.

The blend of phenethyl alcohol and ethylhexylglycerin (sensiva® PA 20) is an efficient stabilization system, combining antimicrobial activity with skin care and deodorizing properties. The synergistic mixture is suitable for preservative-free cosmetics (according to the European interpretation), as well as sensitive applications; e.g. baby care, oral and lip care. Due to the high portion (90 percent) of nature-identical material in this blend, it can be an option to stabilize nature-inspired cosmetics.

### REFERENCES AND NOTES

Our natural cosmetic actives have a high concentration of bioactive components and proven efficacy.

**Follicusan™**
- Counteracts premature, accelerated hair loss (alopecia)
- Improves hair density and thickness
- Vitalizes scalp and hair follicles, stimulates dermal papilla cells
- Milk-based bioactive

**Vitamin F forte**
- Nourishes, revitalizes and strengthens hair
- Hair breakage protection
- Conditions the hair, improving combability
- High-strength ω-6 fatty acids from safflower oil
- NATRUE-certified and conforming to the ECOCERT natural and organic cosmetics standard

**DayMoist CLR™**
- High-performance hair moisturizer
- Protects the hair against color fading
- Improvement of elasticity of the hair
- Conditions hair, improving combability
- Protects hair against heat stress from styling (hair straighteners, dryers)
- Synergistic complex of plant-derived actives
- NATRUE-certified and conforming to the ECOCERT natural and organic cosmetics standard

CLR maintains an integrated management system that combines both quality and environmental aspects in accordance with ISO 9001 and 14001 standards.

**for strong and healthy hair**

CLR
Chemisches Laboratorium
Dr. Kurt Richter GmbH
Berlin • Germany
Tel +49 30-85 10 26-0
info@clr-berlin.com
www.clr-berlin.com

visit us at
in-cosmetics
Barcelona
Stand Q 50