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Emulsifier system influences O/W emulsion preservation

According to the current Cosmetic Regulation EC 1223/2009 a cosmetic product made available on the market should be safe for human health when used under normal or reasonably foreseeable conditions of use. This includes the necessity for an adequate preservation system in the cosmetic product to prevent microorganisms potentially endangering the consumer's health from growing. The preservation of personal care products is achieved by the addition of antimicrobial ingredients. Their properties however bear the potential for undesired effects on the skin. In 1982 a positive list of approved preservatives was adopted as Annex VI to council directive 76/768/EEC,[1] which later turned into Annex V of regulation EC 1223/2009.

In addition to the approved preservatives listed on Annex V, a large number of other personal care ingredients exhibit inherent antimicrobial activity, which may be exploited to complement the overall preservation of a cosmetic product as a secondary function of the ingredients, such as chelators, wetting agents, fragrance ingredients, moisturisers, solvents, antioxidants and others.[2] In fact, most personal care ingredients either have a positive or negative effect on the overall antimicrobial effect of a formulation. Only a few ingredients are inert in the sense that they have absolutely no influence on the preservation of a product.

The nature of the influence of the ingredients on the antimicrobial performance can be manifold. Some ingredients have a negative effect on microorganisms by affecting the cell membrane or the cell metabolism after penetration into the cell, while others stimulate microbiological growth by the ability of the microorganisms to metabolise the ingredient. In addition, interaction between the ingredients may lead to inactivation of antimicrobial active components. Antimicrobial components with surface activity can be attracted by detergent micelle structures in rinse-off formulations or by emulsifiers in the interphase of emulsion systems. A variation of the polarity of the oil-phase has an influence on the partition of antimicrobial ingredients between the water-phase and the oil-phase.

The aim of the investigation reported in this article was to identify and quantify the influence of the emulsifier system on the preservation of the emulsion by microbiological challenge tests. Ideally it will be possible to identify specific structural features or components in the emulsifier composition that show a positive or negative interaction with the preservation performance.

Table 1: General composition of the O/W emulsions.

Phase	Ingredient	INCI (EU)	%
A	Deionised water	Aqua	ad 100
	dermofeel PA-3[1]	Sodium Phytate; Aqua; Alcohol	0.2
	Glycerin (99.5%)[2]	Glycerin	4.0
	dermosoft GMCY[1]	Glyceryl Caprylate	0.5
	dermosoft 700B[1]	Levulinic Acid; Sodium Levulinate; Glycerin; Aqua	0.3
	dermosoft 688 eco[1]	p-Anisic Acid	0.2
	Keltrol CG-RD[3]	Xanthan Gum	0.2
B	***Emulsifier system***	***(see Table 2)***	
	Lanette O[4]	Cetearyl Alcohol	1.5-4.0
	Tegin M[5]	Glyceryl Stearate	2.0
	Sunflower Oil[6]	Helianthus Anuus Seed Oil	4.0
	dermofeel MCT[1]	Tricaprylin	6.0
	dermofeel Toco 70[1]	Tocopherol	0.15
C	Sodium Hydroxide (10%)[7]	Sodium Hydroxide	q.s.
	Citric Acid[7]	Citric Acid	q.s

Suppliers
1 Dr. Straetmans 2 Cremer Oleo 3 CP Kelco 4 BASF 5 Evonik 6 Gustav Hees 7 Merck

Table 2: Emulsifier dosage.

Emulsifier	INCI	%
1	Sodium Cetearyl Sulfate	2.00
2	Potassium Cetyl Phosphate	3.00
3	Glyceryl Stearate Citrate	5.00
4	Polyglyceryl-3 Stearate, Sodium Stearoyl Lactylate	3.00
5	Cetearyl Alcohol, Glyceryl Stearate, Stearic Acid, Sodium Lauroyl Glutamate	6.00
6	Sodium Citrate, Hydrolyzed Milk Protein, Xanthan Gum, Cyamopsis Tetragonoloba (Guar) Gum, Magnesium Stearate	2.50
7	Hydrogenated Lecithin, C12-16 Alcohols, Palmitic Acid	4.00
8	Cetearyl Alcohol, Glyceryl Stearate, Potassium Palmitoyl Hydrolyzed Wheat Protein	5.00
9	Polyglyceryl-3 Methylglucose Distearate	4.00
10	Sorbitan Stearate, Sorbityl Laurate	0.50
11	Cetearyl Alcohol, Cetearyl Glucoside	2.00
12	Cetearyl Glucoside, Cetearyl Alcohol	5.00

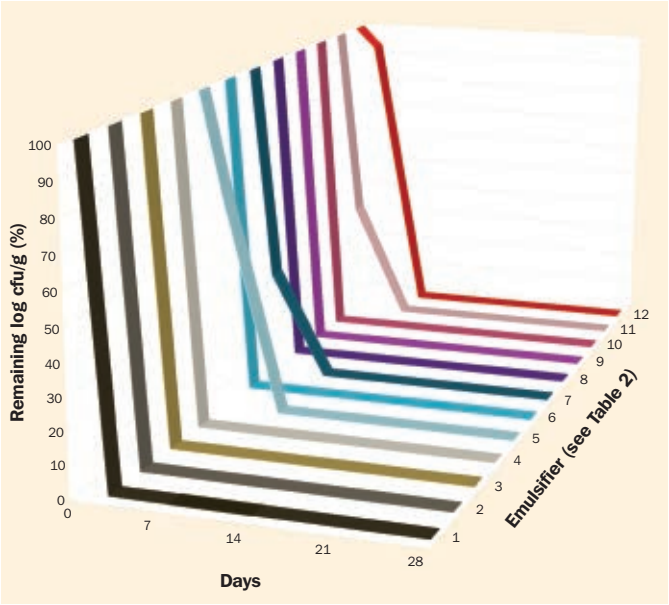

Figure 1: *Challenge test results against* Staphylococcus aureus.

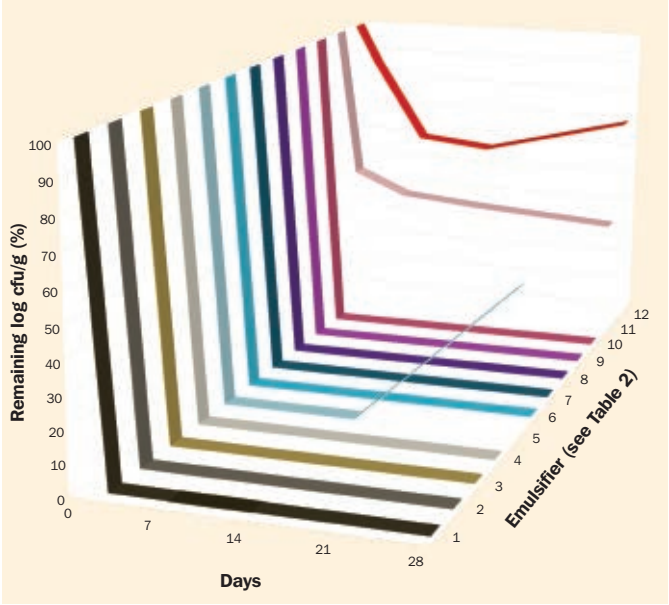

Figure 2: *Challenge test results against* Pseudomonas aeruginosa.

Materials and methods

Twelve different oil-in-water emulsions have been formulated in the laboratory with the general composition shown in Table 1. The emulsifier concentration has been chosen according to the recommendations provided in the suppliers' product documentation. The type of emulsifiers and respective use concentrations are shown in Table 2. In cases where the emulsifier blend already contains fatty alcohol as one of the components, the amount of fatty alcohol in the core formulation has been reduced accordingly to end in all cases with a total concentration of 4% fatty alcohol in the final formulation. The acidity of all formulations has been adjusted to pH 5.3 in order to exclude the influence of a variable pH on the antimicrobial performance of the organic acids.

A standard procedure has been employed to emulsify the water and the oil phase in a hot-hot process. The stability of the emulsions has been assessed by storage at room temperature, 4˚C and 40˚C over a period of three months as well as freeze-thaw-cycles. The stability tests demonstrated sufficient stability for all twelve emulsions. The droplet size and distribution has been checked under a microscope.

Microbiological challenge tests according to the European Pharmacopoeia 3 have been performed with all emulsions. *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 8739), *Candida albicans* (ATCC 12231) and *Aspergillus brasiliensis* (ATCC 16404) were used as microorganisms in this single inoculation test protocol. The plate counts have been performed after 2, 7, 14 and 28 days after inoculation. Plate counts at time zero resulted in between 250,000 and 1,250,000 colony forming units per gram (cfu/g). A typical graph of the results of a single inoculation challenge test will show the reduction of the total number of microorganisms expressed as the logarithmic number of colony forming units, starting with the plate count at time zero. For the display of challenge test results in Figures 1-5 the relative logarithmic reduction is shown, with the plate count at time zero set as 100%. When the plate count at time zero is 1,000,000 cfu/g = 100% (log cfu/g=6), a plate count of

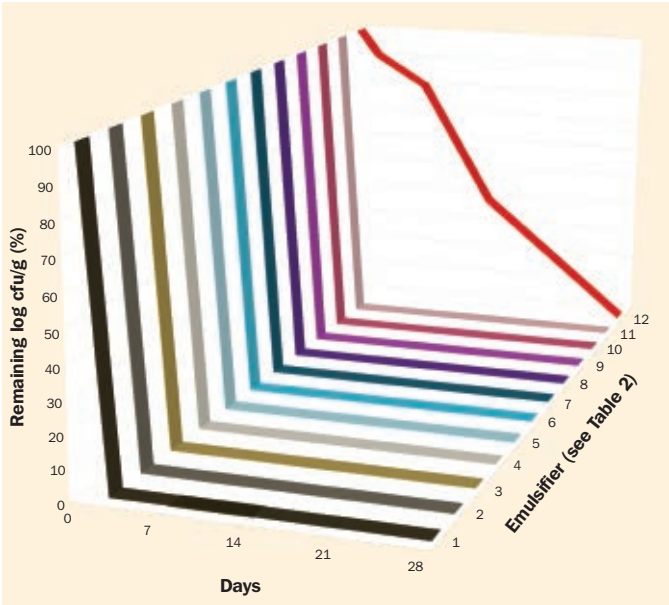

Figure 3: *Challenge test results against* Escherichia coli.

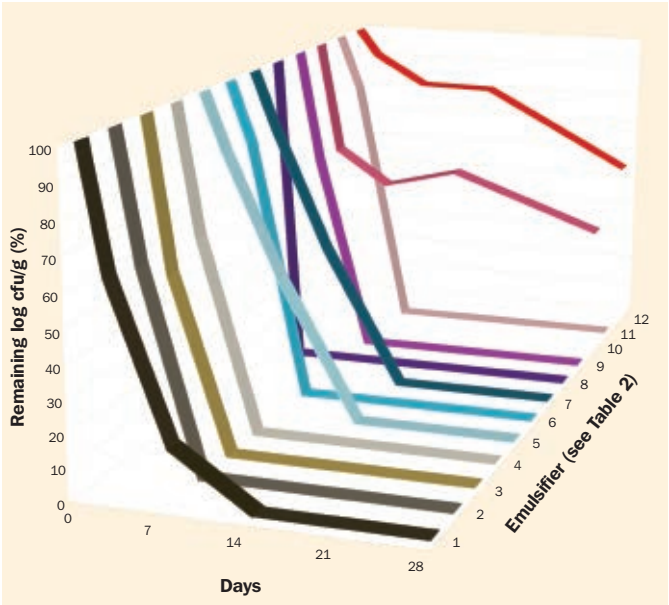

Figure 4: *Challenge test results against* Candida albicans.

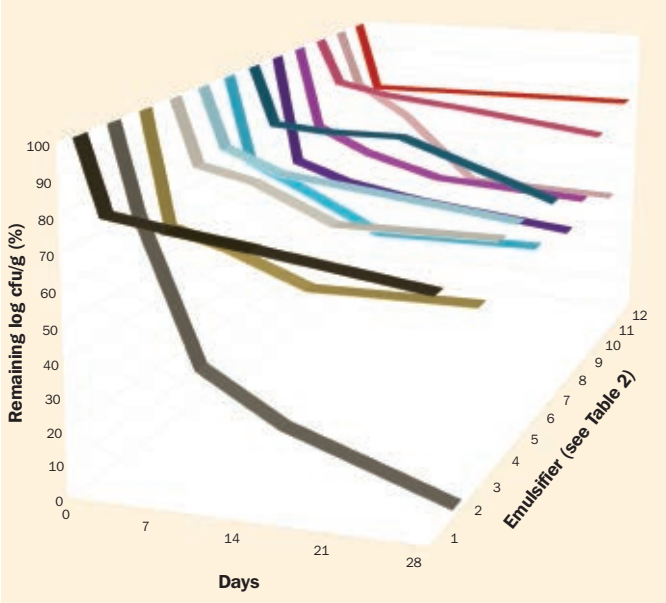

Figure 5: *Challenge test results against* Aspergillus brasiliensis

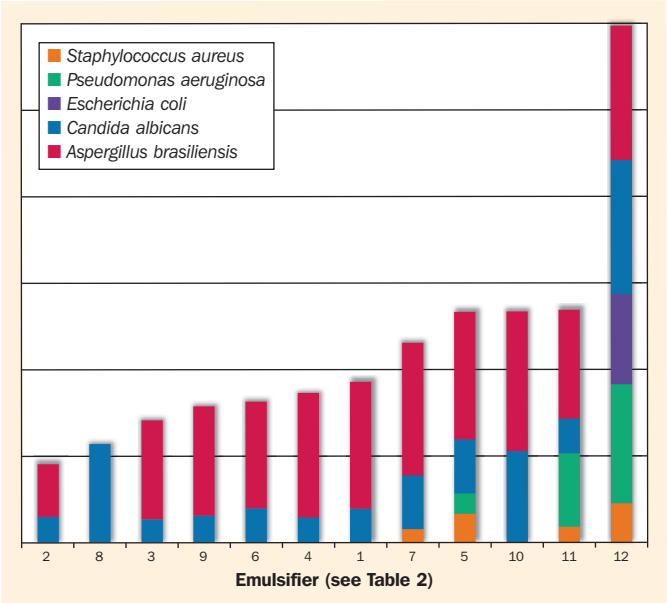

Figure 6: *Relative influence of the emulsifier system on the antimicrobial performance.*

1000 cfu/g (log cfu/g=3) represent 50% relative logarithmic reduction.

Results and discussion

The result of the challenge tests against the five strains of microorganisms are shown in Figures 1-5. The antimicrobial performance of the tested formulations against the bacteria *Staphylococcus aureus* (Fig. 1), *Pseudomonas aeruginosa* (Fig. 2) and *Escherichia coli* (Fig. 3) was too strong to reveal detailed differences between most of the emulsions under the given test conditions, even though differences may exist for the kill rates between the time of inoculation and the first evaluation of plate counts after two days. Kill rate kinetics against fungi are generally slower, allowing a better comparison of the individual emulsions regarding their performance against the yeast *Candida albicans* (Fig. 4) and the mold *Aspergillus brasiliensis* (Fig. 5). In Figure 6 the emulsions are compared with respect to their overall antimicrobial performance. The larger the bar the less favourable is the influence of the emulsifier on the performance of the preservation system.

Emulsifiers are classified into three groups: non-ionic, anionic and cationic. Cationic emulsifiers have not been part of this investigation. However, their potential antimicrobial activity is well established.[3] In the tested series of emulsifiers and emulsifier blends no strict rule can be established that an anionic emulsifier is more favourable than a non-ionic emulsifier with respect to the positive influence on the preservation, but a certain tendency to support this theory can be observed.

Despite the fact that surface active compounds made from hydrocarbon building blocks are known for their antimicrobial properties,[4] the comparison of the results of emulsion 11 and 12 clearly reveals the negative influence of the hydrocarbon related structural feature in this test. Because the level of cetearyl alcohol has been standardised in all formulations, the only difference between emulsion 11 and 12 is the level of cetearyl glucoside. The higher dosage of cetearyl glucoside in emulsion 12 compared to emulsion 11 is further decreasing the antimicrobial performance. Emulsion 10 is the third emulsion in the panel containing hydrocarbon building blocks. Emulsions 10, 11 and 12 are the least preserved emulsions in the test.

The relatively bad test result for emulsion 5 comes as a surprise. It was expected that the component sodium lauroyl glutamate would have a beneficial effect on the preservation performance as a surfactant based on a medium chain fatty acid.[5] The result for emulsion 7 can be explained by the presence of hydrogenated lecithin. Lecithin and polyethoxylated surfactants are used in microbiological laboratories as neutralisers to inactivate the preservatives according to the commonly used test protocols.[6]

Even though the droplet size can be considered as a possible influencing factor due to the potential correlation between the overall surface area of the emulsion droplets and the interaction with antimicrobial ingredients, the challenge test results do not reveal such a correlation.

Conclusions

The emulsifier system showed a pronounced influence on the antimicrobial performance of a surfactant and organic acids based preservation system. Emulsifier blends comprising hydrocarbon moieties showed a negative influence on the antimicrobial performance of the tested preservation system. Anionic emulsifiers have a tendency to support the preservation activity better than non-ionic emulsifiers.

It is important to understand that most ingredients have an influence on the product preservation. This influence can be favourable or detrimental. It cannot be assumed that even a slight change of a formulation or the variation of the dosage of a component does not alter the result of the preservation performance. Challenge tests need to be performed in order to proof sufficient preservation after each change in a formulation composition. PC

References

1 Council Directive 82/368/EEC of 17 May 1982, Article 6.

2 Petersen W. Antimicrobial ingredients for self-preserving cosmetics. *Euro Cosmetics* 1999; **7** (2): 28-36.

3 Cozzoli O. The role of surfactants in self-preserving cosmetic formulas. In: Kabara JJ, Orth DS eds. *Preservative-free and self-preserving cosmetics and drugs*. New York: Marcel Dekker, 1997: 75-118.

4 Matsumura S, Imai K, Yoshikawa S, Kawada K, Uchibori T. Surface activities, biodegradability and antimicrobial properties of n-alkyl glucosides, mannosides and galactosides. *J Am Oil Chem Soc* 1990; **67** (12): 996-1001.

5 Kabara JJ. Structure-function relationships of surfactants as antimicrobial agents. *J Soc Cosmet Chem* 1978; **29**: 733-41.

6 ISO 11930:2012. Cosmetics – Microbiology – Evaluation of the antimicrobial protection of a cosmetic product, Annex C.