Marion Leschke, Wolfgang Siegert – Schülke & Mayr, Germany

Boosting efficacy of preservatives

The topic of preservation is always of importance to formulators and finished goods marketers.

Formulators are aware of the necessity to adequately preserve their products in order to ensure product safety and be in compliance with legislation. This task is made much more difficult when marketing requirements are added to the factors influencing the preservative choice. Demands such as global approval, soft preservation, "free of…", etc have limited the number of acceptable actives.

Increasing marketing pressure has resulted in an interest in reducing the amount of traditional preservatives in cosmetic formulations or in finding novel ways to keep cosmetic products microbiologically stable. It is now more important than ever that preservation or microbiological stability is an integral part of new formulation concepts. At an early stage of product development, formulators must consider all possible methods of enhancing the efficacy of traditional preservative actives, e.g. using chelating agents or multifunctional materials.

There are some ingredients known in the market which can boost the efficacy of preservative actives without having their own antimicrobial effect. Chelating agents are able to enhance the efficacy of most preservatives. This occurs as the chelator removes metal ions from cell walls of the microbes. The weakened walls then allow the biocide to penetrate and destroy the microorganisms. Although the boosting effect of chelating agents on preservatives is well known, the environmental fate of these materials has been debated. To avoid the environmental discussion about chelating agents, readily biodegradable alternatives have been introduced to the market.[1]

Table 1: Formulation tested in challenge test with several preservative blends.

INCI name	Trade name	Content
Glycerine	Glycerine 85%	15.00%
Acrylate crosspolymer	Carbopol ETD 2020	0.40%
Sodium hydroxide	Sodium hydroxide solution, 15% aq.	0.90%
Aqua		ad.100
Preservative (see Table 2 for systems used)		

Table 2: Preservative systems used in challenge test.

Preservative system tested	Content
Phenoxyethanol	0.9%
Phenoxyethanol/ethylhexylglycerin	0.9%/0.1%
Methylisothiazolinone	100 ppm
Methylisothiazolinone/ethylhexylglycerin	100 ppm/0.1%
Methylparaben	0.2%
Methylparaben/ethylhexylglycerin	0.2%/0.1%

Table 3: Chelating agents used to show the effect on the efficacy of preservatives.

Trade name	Active content	INCI name
Baypure CX 100/34%	34%	Tetrasodium iminodisuccinate
Dissolvine GI-38	38%	Tetrasodium dicarboxymethyl glutamate
Octaquest E 30	37%	Trisodium ethylenediamine disuccinate
Trilon B powder	87%	Tetrasodium EDTA

Table 4: Test organisms used in this the model.

Test organisms	ATCC-N°
Escherichia coli	11229
Pseudomonas aeruginosa	15442
Staphylococcus aureus	6538
Candida albicans	10231
Aspergillus niger	6275

Ethylhexylglycerin – a booster for preservatives

Ethylhexylglycerin is a globally approved, versatile and multifunctional additive,[2,3] as well as a very effective deodorant active.[4,5] It is a representative of the 1-alkyl glycerine ethers. It is a high purity, colourless and almost odourless liquid, with excellent properties for use in cosmetic formulations. Ethylhexylglycerin was introduced to the cosmetic market as a skin care additive and deodorant active (sensiva SC 50) in 1992. As a new substance, it is listed on the ELINCS file.

As an emollient and mild humectant, ethylhexylglycerin improves the skin feel of cosmetic formulations. It reliably inhibits the growth and multiplication of odour-causing bacteria, while at the same time not adversely affecting the beneficial skin flora. Additionally, it can boost the efficacy of traditional preservatives[6,7] and act as an antimicrobial stabiliser when used in combination with other cosmetic ingredients.

Ethylhexylglycerin has a surfactant-like structure. Due to this structure, ethylhexylglycerin affects the interfacial tension at the cell membrane of microorganisms, allowing some active ingredients, such as antimicrobials and

preservative actives, to penetrate more effectively.

In order to prove the preservative boosting effect of ethylhexylglycerin, challenge tests have been carried out on a carbomer gel formulation. Table 1 shows the tested formulation. Phenoxyethanol, methylisothiazolinone and methylparaben have been used as preservative actives (Table 2).

Method

Challenge tests have been performed according to the Schülke KoKo test method.[8,9] The Schülke KoKo test is a repeated challenge test designed and validated by Schülke. A mixed suspension of Gram-positive and Gram-negative bacteria, yeasts and moulds is used for six inoculations at weekly intervals. Parallel to each inoculation a sample of the tested product is streaked out onto nutrient media, incubated and evaluated semi-quantitatively. The longer the time before the occurrence of the first microbial growth, the more effective is the preservative. Experience has shown that a well-preserved product should remain growth-free for six inoculation cycles to ensure the shelf-life required in practice (30 months in the original packaging) (Fig. 1).

Results

Challenge test results with the single preservative actives on one hand and the combination of the single actives with ethylhexylglycerin on the other are listed in Figure 2. The results impressively show the boosting effect of 0.1 % ethylhexylglycerin on the preservation effect of phenoxyethanol, methylisothiazolinone and methylparaben. While the single actives fail after 2 inoculation cycles, the addition of ethylhexylglycerin increased the efficacy of phenoxyethanol, methylisothiazolinone and methylparaben sufficiently to pass the KoKo test.

The combination of phenoxyethanol and ethylhexylglycerin is well-described in literature.[6,7] It is protected by patent and commercially available under the trade name euxyl PE 9010. The combination with methylisothiazolinone is also protected by patent and supplied in the market as euxyl K 220.

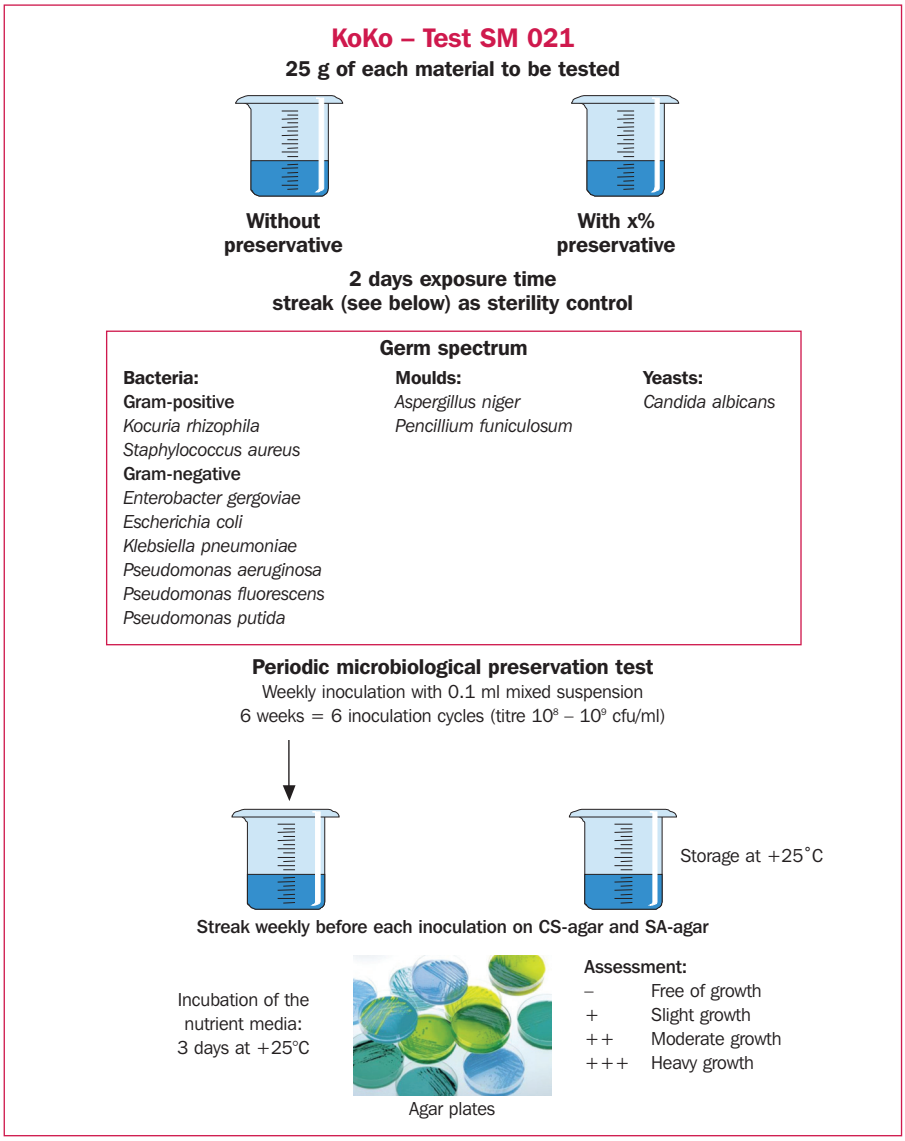

Figure 1: *Evaluation of the microbial stability of cosmetic formulations with the Schülke KoKo test.*

Boosting preservative efficacy with chelating agents

While in the past there were discussions about the environmental effect of chelating agents, new readily biodegradable alternatives have been developed and tested under reproducible conditions. Several examples of this new generation of chelating agents have been tested *vs* traditional tetrasodium EDTA regarding their ability to increase the efficacy of cosmetic preservatives. For the following series, euxyl PE 9010 has been chosen as the test preservative.

To obtain a clear differentiation between the preservative with and without complexing agent, the use concentration of the preservative was selected at the lowest effective concentration. For the combination phenoxyethanol/ethylhexylglycerin (euxyl PE 9010) in water this is 0.75 %. Different chelating agents dosed at 0.1% and 0.2% active material have been tested (Table 3).

Method

Dilutions of the preservative and combinations of the preservative/booster are prepared using sterile hard water according to the European standard for testing chemical disinfectants and antiseptics.[10] Worked with are 50 ml quantities of the end solutions and these quantities are each inoculated with 0.5 ml microorganism suspension (initial microorganism count approx 10^8 cfu/ml) and stirred. Table 4 shows the test organisms used.

These solutions are streaked out onto tryptone soya agar or sabouraud-dextrose 4% agar after 1, 3, 6, and 24 hours. The cultures are incubated for 48 hours at 37˚C, except for *Aspergillus niger*, which is incubated for 72 hours at 25˚C–27˚C. The evaluation is made on the basis of semi-quantitative assessment of the microbial growth of the streaks.

Due to the alkalinity of the complexing agents, the final solution is adjusted to pH 7.0. Hydrochloric acid (HCl) is chosen as inorganic acid and citric acid as organic alpha-hydroxy acid. The citrates formed can support the chelating effect.

Results

The graphs in Figure 3 show the boosting effect of the chelating agents on euxyl PE 9010.

All of the complexing agents used in this

Carbomer gel	Inoculation cycles						
	0	1	2	3	4	5	6
Without preservation	–	+++ B, M	+++ B, M	./.			
+ 0.1% ethylhexylglycerin	–	+++ M	+++ B, M	./.			
+ 0.9% phenoxyethanol	–	+++ B	+++ B, Y	./.			
+ 0.9% phenoxyethanol + 0.1% ethylhexylglycerin	–	–	–	–	–	–	–
+ 100 ppm methylisothiazolinone	–	+++ M	+++ B, M	./.			
+ 100 ppm methylisothiazolinone + 0.1% ethylhexylglycerin	–	–	–	–	–	–	–
+ 0.2% methylparaben	–	+++ B, Y	+++ B, Y	./.			
+ 0.2% methylparaben + 0.1% ethylhexylglycerin	–	–	–	–	–	–	–
Legend:	0 = Sterility control B = Bacteria M = Moulds Sp = Spore-forming bacteria Y = Yeasts			– = Free of microbial growth + = Slight growth ++ = Moderate growth +++ = Massive growth			

Figure 2: *Schülke KoKo test results showing the preservative-boosting effect of ethylhexylglycerin.*

test exhibit a boosting effect on the preservative. Noticeably, tetrasodium dicarboxymethyl glutamate in combination with citric acid gives a substantially better effect than can be achieved with any of the other combinations tested. Particularly unexpected is the good effect of tetrasodium dicarboxymethyl glutamate in combination with citric acid against fungi.

Practical experience

In order to transfer these results into practical cosmetic systems, additional Schülke KoKo tests have been conducted. One example is presented in Figure 4.

A sun care product has been tested containing 1.0% euxyl PE 9010 with and without the addition of a chelating agent. In this case 0.2% disodium EDTA (Trilon BD) was used.

The results are very clear. While the sun care product with euxyl PE 9010 alone failed in the Schülke KoKo test, after addition of the chelating agent the product was well preserved for 6 inoculation cycles.

Conclusion

Ongoing public discussions about several preservative actives and the increasing demands of marketing make it now more important than ever to find ways to boost the efficacy of traditional preservative actives.

Ethylhexylglycerin has been proven to increase the efficacy of preservative actives such as phenoxyethanol, methylisothiazolinone or methylparaben. Furthermore, it has been shown that chelating agents can have an additional boosting effect on preservative/ethylhexylglycerin blends.

This innovative combination of ethylhexylglycerin and chelating agent may help to reduce the quantity of preservatives required to adequately protect cosmetic formulations. PC

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10 European Standard (CEN), Chemical disinfectants and antiseptics.

Natural sunscreen SPF 30	Inoculation cycles						
	0	1	2	3	4	5	6
Without preservation	–	+++ B, Y, M	+++ B, Y, M	./.			
+ 1.0% euxyl PE 9010	–	+++ B, Y, M	+++ B, Y, M	./.			
+ 1.0% euxyl PE 9010 + 0.2% disodium EDTA	–	–	–	–	–	–	–
Legend:	0 = Sterility control B = Bacteria M = Moulds Sp = Spore-forming bacteria Y = Yeasts			– = Free of microbial growth + = Slight growth ++ = Moderate growth +++ = Massive growth			

Figure 4: *Sun care product preserved with euxyl PE 9010, with and without disodium EDTA.*

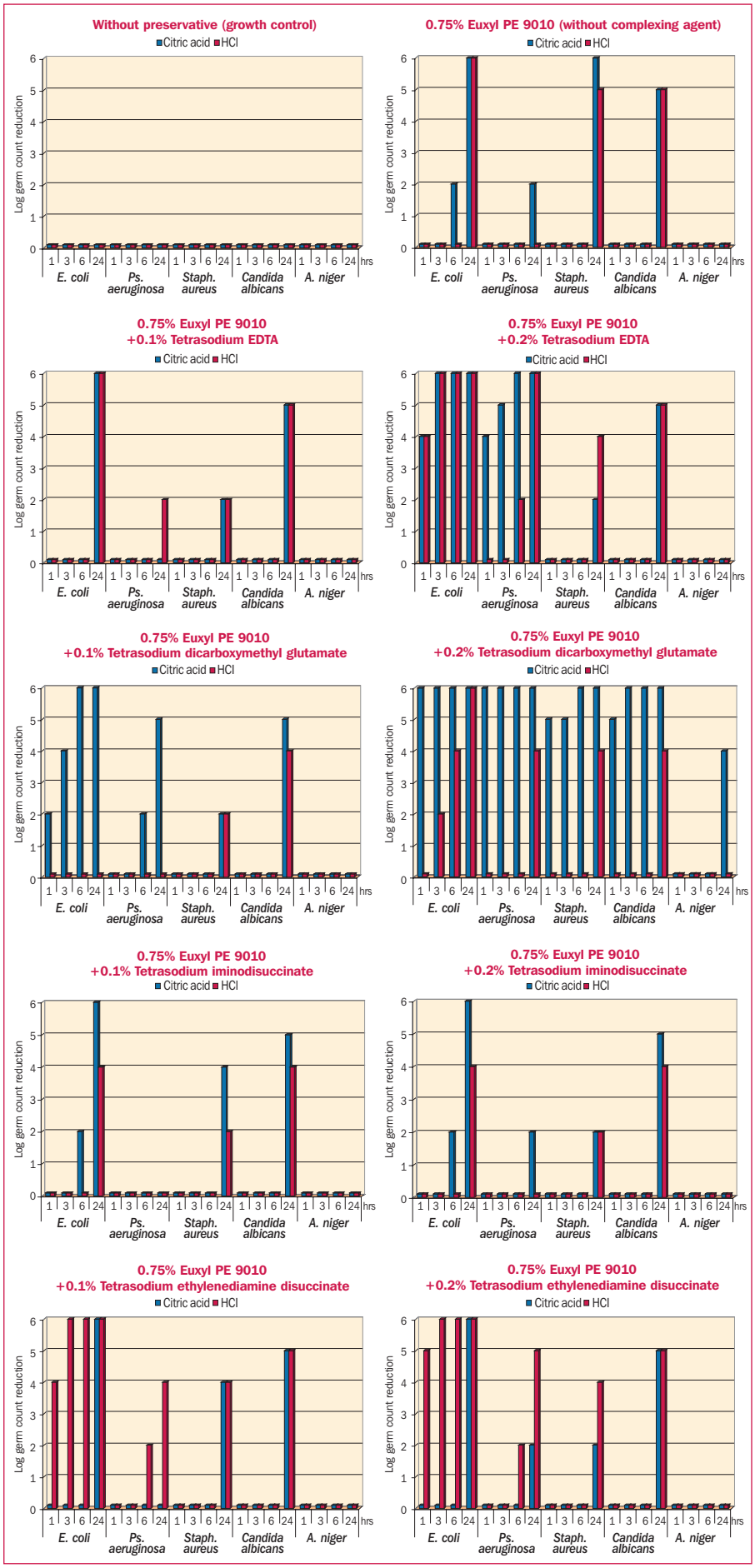

Figure 3: *Test results showing the boosting effect of different chelating agents on the antimicrobial efficacy of euxyl PE 9010.*