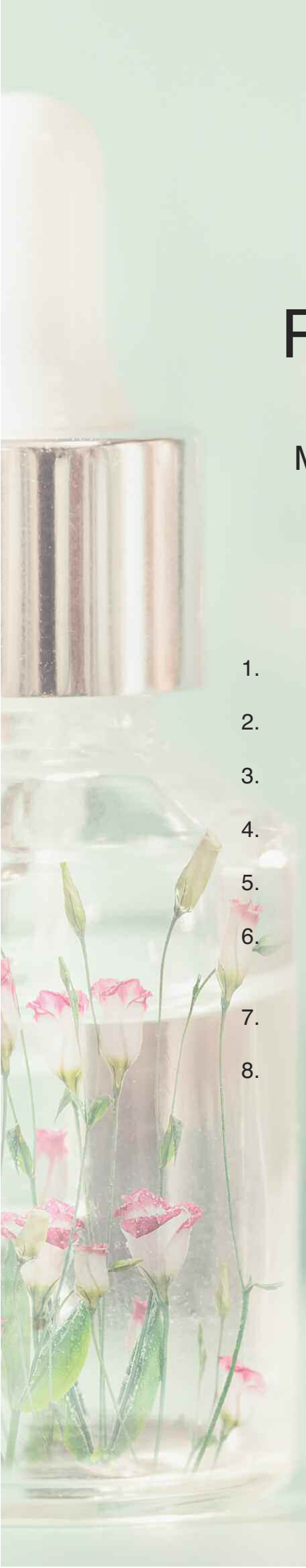
Overview of

NATURAL Preservatives

Compiled by Lise Andersen
& Rebecca Wright



OVERVIEW OF

NATURAL PRESERVATIVES

MANUFACTURER INFORMATION & Articles

1. Dermosoft 1388 by Dr. Straetmans
2. Geogard ECT by Lonza
3. Geogard Ultra by Lonza
4. Iscaguard PFA by ISCA
5. Lexgard Natural by Inolex
6. Sorbic Acid and Potassium Sorbate as Cosmetic Preservatives by Eastman
7. Spectrastat by Inolex
8. Article in Journal of Applied Microbiology: Weak-Acid Preservatives

Compiled by
Rebecca Wright & Lise M Andersen
for members of
Natural Cosmetic Formulating & Formulators Kitchen

Hello!

The most frequent question we get from members of
Natural Cosmetic Formulating Group
and
Formulators Kitchen
is
“Which is the best natural preservative?”

We wish there was a short and simple way to answer this, but there isn’t.
Choosing the right preservative is always formula specific.

This compliation of articles
and manufacturer information on some preservatives
that are accepted as ‘natural’
is our best way of answering this question.

For specifics on preservatives (dosage, use, pH etc),
we recommend starting with the manufacturer’s information.

We hope this will be useful as a guideline and aid
in helping you make an informed decision
on which preservative you use.

Enjoy!

[M]BOTANICALS

Rebecca Wright

Lisa Lise

Lise M Andersen

Dermosoft 1388 by Dr. Straetmans

Multifunctional Additives

Product Information

Dermosoft® 1388

Product features:

- Multifunctional fragrance composition
- Moisturizing effect
- Skin friendly
- Anti-inflammatory properties
- Broad antimicrobial activity
- For various cosmetic formulations

drstraetmans
intelligence behind beauty

Dermosoft® 1388

Dermosoft® products cover many cosmetic functions

The product line

Dermosoft® products are carefully chosen multifunctional cosmetic ingredients. The well balanced product profiles are tailored to the needs of cosmetic formulations. Basic cosmetic functions like hydrating, conditioning, masking and others are combined with an excellent antimicrobial profile. Dermosoft products will meet many of your requirements for the improvement of cosmetic formulations and along the way protect the product against microorganisms. With the aid of Dermosoft® cosmetic products can easily be formulated without traditional preservatives.

Dermosoft® 1388 features natural antimicrobial activity

Dermosoft® 1388

The product's active principle is a blend of compounds found in many plants in nature. In combination with plant derived glycerol contained in this skin friendly mixture a moisturizing effect can be created. The delicate scent of Dermosoft® 1388 will help to mask undesired odours of raw materials but will usually not interfere with other fragrance. The gently acidic ingredients will improve the natural acidic environment of the skin. And finally, the outstanding antimicrobial activity of Dermosoft® 1388 can convert most cosmetic formulations in self preserving products – with no further need for traditional preservatives. Interestingly also bees use one of the contained natural acids for the difficult task of protecting their nest provisions (pollen and nectar) against microbiological spoilage[1].

Efficacy and easy application are the cornerstones of Dermosoft® 1388

Application

In order to further improve the versatility of these products we also focussed on the convenience of our Dermosoft® range. Dermosoft® 1388 is liquid and clearly water soluble and can be employed easily in surfactant based rinse off concepts, emulsions (O/W an W/O) as well as in hydroalcoholic products. To avoid recrystallization and maximum efficacy please regard the recommended use level and the pH requirements.

As a result of our product development Dermosoft® 1388 provides:

- easy application
- compatibility with cold processes
- broad spectrum microbiological activity

Characteristics of Dermosoft® 1388				
Appearance	Clear, colourless to pale yellow liquid			
INCI	Parfum			
Recommended dosage	3,0 – 4,0 %			
Antimicrobial performance ●● very good ● fair ●/○ moderate ○ not sufficient	Gram+	Gram-	Yeast	Mould
	●●	●●	●	●●
pH-range	4,5 - 5,5			
Regulatory status	Registered in EU, US, Japan			

Dermosoft® 1388

Cosmetic functions

Different cosmetic functions are obtained with Dermosoft® 1388

Hydrating
The hydrating effect of glycerine has been proved in many clinical studies[2] and has long been used in cosmetic formulations. It's efficacy has been shown to supersede the hydrating capacity of urea or propylene glycol[3]. The amount of glycerol contained in Dermosoft® 1388 will contribute to the hydrating properties of the cosmetic product at recommended use concentrations.

Masking
The perfume ingredients in Dermosoft® 1388 are known as masking agents. The unspecific scent does not make them first choice for use as a stand alone perfume. But the aroma is appreciated by many formulators to mask undesired odours of raw materials. The light smell will usually not add to or interfere with the perfume in the product.

Acidifying
Dermosoft® 1388 contains two organic acids that are found in nature in many plants. As an intrinsic property of such organic acids the acidity is very low. This makes them ideal candidates for a gentle acidifying effect on human skin. Thus the natural acidic level of the skin can be maintained for a longer time. The correlation between physiological pH and healthy skin has been shown in many studies and there has been evidence that micro organisms like Propionibacterium acne and Staphylococcus aureus and even viruses are significantly reduced, by organic acids and when the normal pH on human skin is maintained stable[4,5].

Anti-inflammatory
Dermosoft® 1388 contains a compound with known anti-inflammatory effect that can act soothing on irritated skin. The anti-inflammatory effect of this compound has been shown to be comparable to other agents like phospholipid analogues, sterols, or vitamin E analogues[6].

Antimicrobial efficacy
Although Dermosoft® 1388 may be employed for many of its additional valuable cosmetic functions, the excellent antimicrobial activity will very well improve the microbiological stability. In most cases it will allow to eliminate unnecessary preservatives from the product. As can be seen in the following figures all relevant germs are destroyed quickly and effectively. The blend contains one compound with bactericidal properties while the co-active shows excellent fungicidal action. Together the two actives display a very good and broad anti microbial performance. For an optimum efficacy the pH of the formulation should not be higher than 5,5.

Dermosoft® 1388

All the microbiological tests are done in an independent external and certified laboratory according to the Pharmacopoeia Europea. The following examples show test results of challenge tests with state of the art products that contain Dermosoft®.

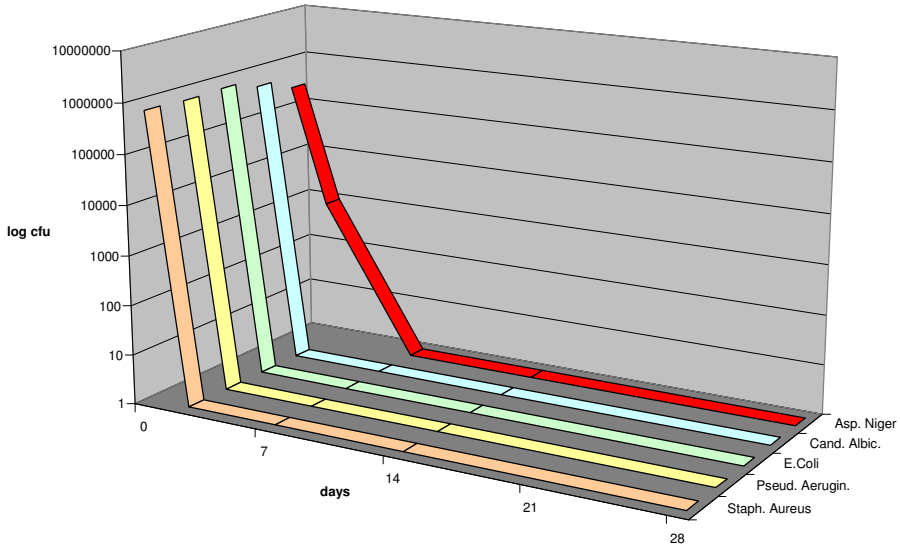


Figure 1: Challenge Test with Shampoo Baby Care stabilized with 3,5 % Dermosoft® 1388

Many cosmetic formulations can be stabilised with Dermosoft® 1388

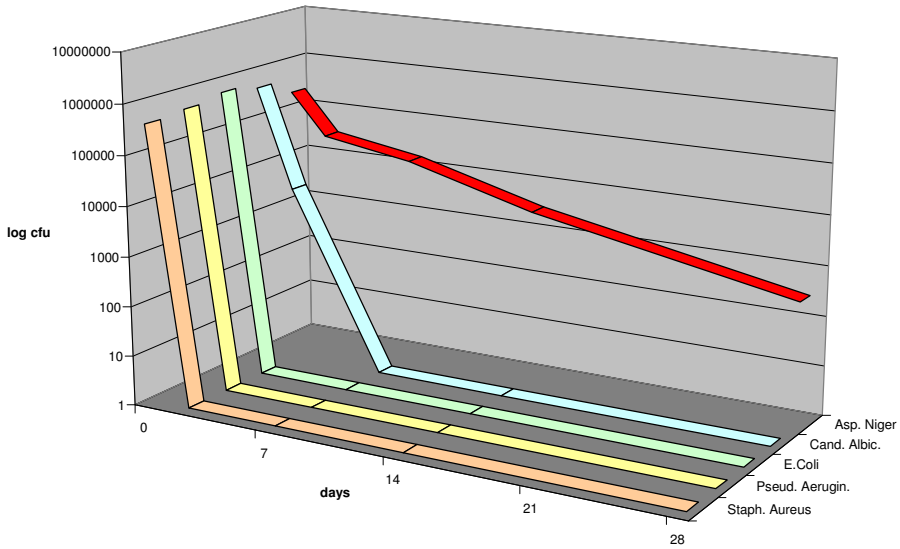


Figure 2: Challenge Test with Skin Serum stabilized with 2,25 % Dermosoft® 1388

Dermosoft® 1388

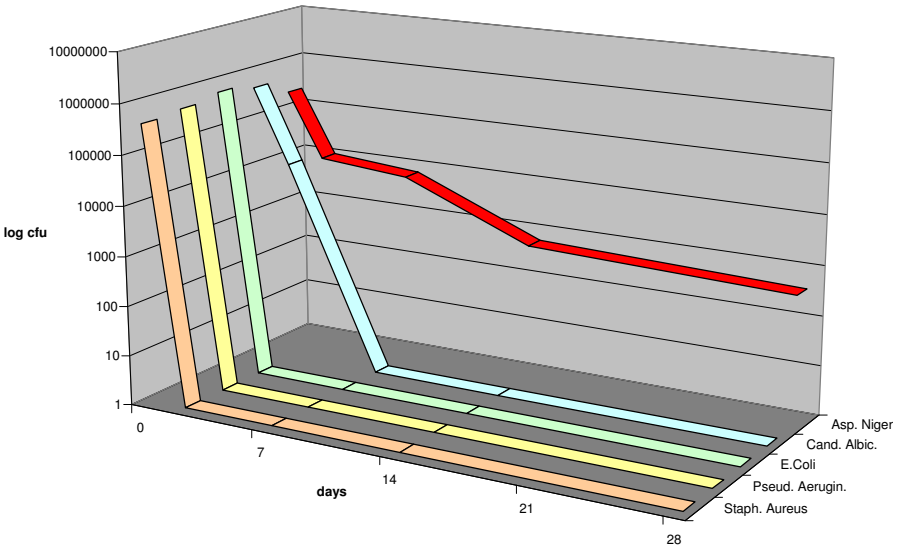


Figure 3: Challenge Test with Organic Body Lotion stabilized with 3,0 % Dermosoft® 1388

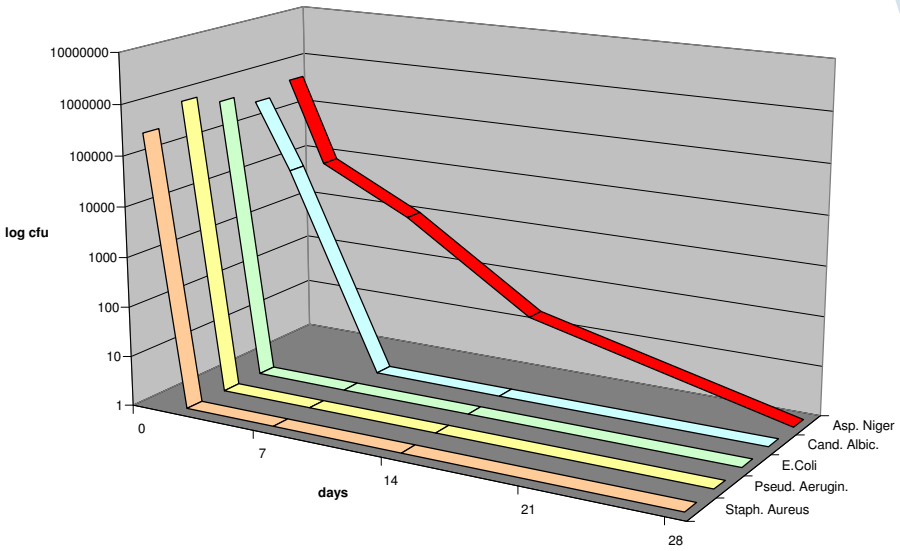


Figure 4: Challenge Test with rinse off Hair Conditioner stabilized with 3,0 % Dermosoft® 1388

The combination of mild masking agents in a skin friendly and moisturizing solution form our Dermosoft® 1388. This furnishes your formulation with a reliable biological stabilization. Just add Dermosoft® 1388 to your formulation and adjust the pH to the recommended level. Using Dermosoft® has never been easier!

Dermosoft® 1388

Skin Care Cream

P11-GSC-8

Claims: **Preservative free**
PEG-free

Phase	Ingredient	INCI	Supplier	%
A	Deionised Water	Aqua		67.50
	Dermofeel® PA-3	**Sodium Phytate, Aqua**	**Dr. Straetmans**	**0.10**
	Glycerol	Glycerin		3.00
	Dermosoft® 1388	**Glycerin, Aqua, Parfum**	**Dr. Straetmans**	**3.00**
	Dermosoft® GMCY	**Glyceryl Caprylate**	**Dr. Straetmans**	**0.30**
A1	Keltrol T	Xanthan Gum	CP Kelco	0.50
	Laracare A 200	Galactoarabinan	Larex	0.20
B	**Dermofeel® GSC**	**Glyceryl Stearate Citrate**	**Dr. Straetmans**	**3.50**
	Dermofeel® SL	**Sodium Stearoyl Lactylate**	**Dr. Straetmans**	**1.50**
	Miglyol 812	Caprylic/Capric Triglyceride	Sasol	3.00
	Dermofeel® Toco 70	**Tocopherol, Helianthus Annuus (Sunflower) Seed Oil**	**Dr. Straetmans**	**0.10**
	PCL Liquid 100	Cetearyl Ethylhexanoate	Symrise	3.00
	Dermofeel® BGC	**Butylene Glycol Dicaprylate/Dicaprate**	**Dr. Straetmans**	**3.00**
	Lanette 16	Cetyl Alcohol	Cognis	4.00
	Cutina GMS	Glyceryl Stearate	Cognis	3.00
	DC 556	Phenyl Trimethicone	Dow Corning	0.10
	Sunflower Seed Oil	Helianthus Annuus		4.00
C	Parf. Waterlily 446652 (SCCNFP-sensitizer free up to 10% use-level)	Parfum	Symrise	0.20
				100.00

Manufacturing Procedure:

1. Heat phase A up to 78 °C.
2. Disperse Keltrol and Laracare one after the other until completely dissolved.
3. Heat phase B to 78 °C. Emulsify phase B to phase A under stirring.
4. Homogenize for 1-2 min. using an Ultra Turrax.
   Start to cool down to 32 °C under stirring. Add perfume oil.

Specification Values:

Appearance: viscous cream.
pH: 4.8 – 5.4.
Viscosity (Brookfield: Helipath TF; Speed 10): approx. 80 000 mPa·s.
Centrifugation (4000 rpm, 15 min.): no separation.

Microbiological Stability: proven.

Dermosoft® 1388

Color Saver Shampoo

L013-24-307

Claims*: Supports a lasting, shiny hair color
Reduces combing force without loss of volume
Leaves a protective film on the cuticle
Preservative free

Phase	Ingredient	INCI	Supplier	%
	Tap Water	Aqua		56.90
	Dermofeel® PA-3	**Sodium Phytate, Aqua**	**Dr. Straetmans**	**0.10**
	Dermosoft® 1388	**Glycerin, Aqua, Parfum**	**Dr. Straetmans**	**3.00**
	D-Panthenol	Panthenol	BASF	1.00
	Texapon N 70	Sodium Laureth Sulfate	Cognis	16.00
	Tego Betain F 50	Cocamidopropyl Betaine	Degussa	10.00
	Lamesoft PO 65	Coco Glucoside, Glyceryl Oleate	Cognis	2.00
	Frag. 4879886 Color Impulse	Parfum	Drom	0.50
	Gluadin AGP	Hydrolyzed Wheat Gluten, Hydrolyzed Wheat Protein	Cognis	0.20
	Euperlan PK 1200	Coco-Glucoside, Glycol Distearate, Glycerin	Cognis	7.50
	Amylomer® 25 L	**Starch Hydroxypropyltrimonium Chloride**	**Dr. Straetmans**	**2.00**
	Amylomer® H 75 M	**Hydroxypropyl Oxidized Starch PG-Trimonium Chloride**	**Dr. Straetmans**	**0.80**
	Sodium Chloride	Sodium Chloride		q. s.
	Citric Acid (sol.20%)	Citric Acid	Merck	q. s.
				100.00

Manufacturing Procedure:
Mix ingredients in given order under stirring until completely dissolved. Adjust viscosity with sodium chloride and pH with citric acid.

Specification Values:
Appearance: White pearlescent viscous gel.
pH: 5.2 – 5.4
Viscosity (Brookfield: LV 3; Speed 10): 3000 – 6000 mPa·s.

Microbiological stability: proven.

*** Claims supported by combing force- and UV-measurements**

Dermosoft® 1388

Rinse off Hair Mask

84-08-906

Claims: Ideal Conditioner for normal to slightly porous hair
Long term conditioning effect
Smooth, well conditioned feel
Gives a nice slip, covers roughness of porous hair

Phase	Ingredient	INCI	Supplier	%
A	Tap Water	Aqua		76.90
	Jaguar HP 105	Hydroxypropyl Guar	Erbslöh	0.35
A1	**Dermofeel® PA-3**	**Sodium Phytate**	**Dr. Straetmans**	**0.10**
	Dermosoft® 1388	**Glycerin, Parfum, Aqua**	**Dr. Straetmans**	**3.00**
B	Incroquat Behenyl TMS	Cetyl Alcohol, Behentrimonium Methosulfate	Croda	7.00
	DC 200 (100 cs)	Dimethicone	Dow Corning	0.50
	Dermofeel® BGC	**Butylene Glycol Dicaprylate/Dicaprate**	**Dr. Straetmans**	**6.50**
D	**Amylomer® EMU**	**Polyquaternium-75**	**Dr. Straetmans**	**3.75**
	Amylomer® 25 L	**Starch Hydroxypropyltrimonium Chloride**	**Dr. Straetmans**	**1.60**
E	Parf. Leafs in the trees 419475 (SCCNFP sensitizer free up to 10% use level)	Parfum	Symrise	0.30
	Citric Acid (sol 20%)	Citric Acid	Merck	q. s.
				100.00

Manufacturing Procedure:
1. Dissolve Jaguar HP 105 under stirring while heating to 75°C.
2. Add components of phase A1.
3. Heat phase B up to 75°C. Add phase B to phase A under stirring.
4. Homogenize for 1-2 min. using an Ultra Turrax.
5. Cool down to 40°C. Add Amylomer and Parfum under stirring.
6. Adjust pH value to 5,0 – 5,4.

Specification Values:
Appearance: white emulsion.
pH: 4.5 – 5.0.
Viscosity (Brookfield Spin 6; Speed 10): approx. 20.000 mPa·s.
Centrifuge (10 min., 4000 rpm): no separation.

Stability:
stable for more than 3 month at 20°C, 40°C, and 4°C

Microbiological Stability: proven.

* distributed in Germany by Dr. Straetmans GmbH

Dermosoft® 1388

Skin Normalizing Cream

63-03-1205

Claims: Preservative free
Sebum regulating
PEG-free

Phase	Ingredient	INCI	Supplier	%
A	Deionised Water	Aqua		58.45
	Glycerol	Glycerin		2.00
	Dermosoft® GMCY	**Glyceryl Caprylate**	**Dr. Straetmans**	**0.50**
	Dermosoft® 1388	**Glycerin, Aqua, Parfum**	**Dr. Straetmans**	**2.00**
A1	Keltrol RD	Xanthan Gum	Kelco	0.30
B	**Dermofeel® SL**	**Sodium Stearoyl Lactylate**	**Dr. Straetmans**	**2.00**
	Dermofeel® PS	**Polyglyceryl-3 Stearate**	**Dr. Straetmans**	**2.00**
	Dermofeel® BGC	**Butylene Glycol Dicaprylate/Dicaprate**	**Dr. Straetmans**	**6.00**
	Dermofeel® Toco 70	**Tocopherol, Helianthus Annuus (Sunflower) Seed Oil**	**Dr. Straetmans**	**0.05**
	Miglyol 812	Caprylic/Capric Triglyceride	Sasol	8.00
	Cutina GMS	Glyceryl Stearate	Cognis	2.00
	Lanette O	Cetearyl Alcohol	Cognis	4.00
C	Deionised Water	Aqua		10.00
	Linumine	**Linum Usitatissimum (Linseed) Seed Extract**	**Lucas Meyer***	**1.00**
D	Ethanol	Alcohol denat.		1.00
E	Citric Acid (sol 20%)	Citric Acid	Merck	0.45
	Parf. Baby Cotton 449264 (free of SCCNFP sensitizer)	Parfum	Symrise	0.25
				100.00

Manufacturing Procedure:

1. Heat phase A up to 75°C. Disperse Keltrol until completely dissolved.
2. Heat phase B up to 75°C. Emulsify phase B to phase A under stirring. Homogenize if necessary.
3. Cool down to 50°C and add pre-solution of phase C.
4. Cool further down below 30°C and add part D.
5. Add Perfume and adjust pH with citric acid.

Specification Values:

Appearance: viscous, light-brown cream.
pH: 5.0 – 5.4.
Viscosity (Brookfield: Spindle 6; Speed 10): approx. 25 000 mPa·s.
Centrifugation (5000 rpm, 2900 RZB, 10 min.): no separation.

* distributed in Germany by Dr. Straetmans GmbH

Microbiological Stability: proven.

Dermosoft® 1388

Dozens of formulation examples are compiled in our Formulary

More formulations with our products are available for both, traditional and natural cosmetics concepts. Please contact us to receive your copy of our general Formulary and our Formulary NATURE Edition, respectively.

Toxicology

Dermosoft® 1388 is not irritating, not sensitizing and does not contain genetically modified material, dioxine, phthalates, BSE-related material or CMR-material. Without the 26 sensitizers, it is in full compliance with the IFRA codes and the 7th amendment.

Packing units

Dermosoft® 1388 is available in 10 kg and 25 kg canisters and in 200 kg drums.

Environmental Information

Dermosoft® 1388 is made from environmentally and toxicologically unobjectionable raw materials. Dermosoft® 1388 is fully biodegradable and has not been tested on animals.

Handling and storage

In closed original containers Dermosoft® 1388 can be stored for at least 3 years. Dermosoft® 1388 does not need to be preserved.

[1] Vinson SB, et al., Nest liquid resources of several cavity nesting bees in the genus Centris and the identification of a preservative, levulinic acid,. *J Chem Ecol.* 2006; 32(9): 2013-21.

[2] Bettinger J, et al., Opposing Effects of Glycerol on the Protective Function of the Horny Layer against Irritants and on the Penetration of Hexyl Nicotinate. *Dermatology* 1998;197:18-24.

[3] Bettinger J, et al., Comparison of different non-invasive test methods with respect to the effect of different moisturizers on skin, *Skin Research and Technology*, 1999, 5 (1), 21–27.

[4] Turner RB, et al., Efficacy of Organic Acids in Hand Cleansers for Prevention of Rhinovirus Infections, *Antimicrobial Agents And Chemotherapy*, 2004, p. 2595–2598 Vol. 48, No. 7

[5] Schmidt-Wendtner MH, Korting HC, The pH of the Skin Surface and Its Impact on the Barrier Function, *Skin Pharmacol Physiol* , 2006;19:296–302

[6] Singh N, et al., Crystal Structures of the Complexes of a Group IIA Phospholipase A_2 with Two Natural Anti-inflammatory agents, Anisic Acid, and Atropine Reveal a Similar Mode of Binding, *Proteins*, 2006; 64: 89–100

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drstraetmans
intelligence behind beauty

Geogard ECT by Lonza

Personal Care

Lonza

Consumer Care

Geogard® ECT (patented)

Broad Spectrum Preservation System

INCI Name: Benzyl Alcohol & Salicylic Acid & Glycerin & Sorbic Acid
SAP Code#: 139650

Key Product Attributes:

- A preservation system that meets the ECOCERT standards
- COSMOS accepted
- Broad spectrum activity on bacteria, yeast and molds
- Has a wide range of global regulatory acceptance* †
- Low odor profile; Ideal for fragrance-free and fragrance-sensitive systems
- Compatible in a wide range of skin care, hair care and sun care systems
- Wide pH compatibility: pH 3 – 8
- Excellent safety profile

** In Europe, there are restrictions in using Salicylic Acid in products for children under the age of 3.*

† In Japan, Benzyl Alcohol is not an approved cosmetic preservative, however it can be used as a cosmetic ingredient.

Recommended Use Level

0.6 – 1.0%

Description

Geogard® ECT is a unique, patented combination of 4 components: Benzyl Alcohol, Salicylic Acid, Sorbic Acid, and Glycerin, which are well-accepted in a wide range of personal care products. The novel composition of this antimicrobial blend offers broad spectrum protection in a diverse range of products against Gram-positive & Gram-negative bacteria, yeast and molds.

Compositional Breakdown

Chemical Compound Breakdown	CAS No.	EINECS
Benzyl Alcohol	100-51-6	202-859-9
Salicylic Acid	69-72-7	200-712-3
Glycerin	56-81-5	200-289-5
Sorbic Acid	110-44-1	203-768-7

Chemical Compositional Breakdown	%
Benzyl Alcohol	77-86%
Salicylic Acid	8-15%
Glycerin	3-5%
Sorbic Acid	1-4%

Applications

- Anhydrous
- Body Butter
- Body wash
- Conditioner
- Cream
- Deo/ Anti-Perspirant
- Eye creams/gels
- Eye shadow
- Face Lotion
- Face wipes
- Facial Cream
- Foundation
- Hair gel
- Hand soap (non anti-bac)
- Liptick/gloss
- Lotion
- Make up remover
- Mascara
- Oil in Water
- Oral care
- Powder
- Shampoo
- Suncare
- Toner
- Vaginal (exterior)
- Water in Oil

Efficacy

Microbiological Challenge Studies

Studies were run on five formulas using a 1.0% concentration of Geogard® ECT. The protocol used was a CTFA challenge test. All samples were inoculated at the beginning of the study, sampled at 24 hours, 7, 14, 21 and 28 days. The samples were diluted in neutralizer and plated quantitatively for viable organisms at all sampling times. After 28 days, all samples were re-inoculated and subjected to a second challenge.

Make-Up Remover

pH: 5.15
% water: 90%; A_w: 0.980

Ingredient	%
Deionized Water	q.s. to 100%
Propylene Glycol	2.00%
Glycerin	2.00%
PEG-8	2.00%
Decyl Glucoside	4.00%
Total	100.00%

Test Results

Colony Forming Units per Gram (CFU/g)

Test Organism	Unpreserved Control				Test-Geogard® ECT (1%)			
	Initial Challenge			Rechallenge	Initial Challenge			Rechallenge
	24 hrs	7 days	28 days	28 days	24 hrs	7 days	28 days	28 days
S. aureus	$9.0x10$	<10	<10	<10	$2.0x10$	<10	<10	<10
K. pneumoniae + *E. gergoviae*	$5.3x10^3$	<10	<10	<10	$4.0x10$	<10	<10	<10
P. aeruginosa + *B. cepacia*	$3.3x10^5$	$1.8x10^6$	$1.4x10^6$	$7.7x10^6$	$1.0x10$	<10	<10	<10
C. albicans	$1.8x10^4$	$1.9x10^4$	$1.2x10^4$	$1.5x10^4$	<10	<10	<10	<10
Mixed molds	$1.5x10^4$	$2.4x10^4$	$1.1x10^4$	$7.0x10^4$	<10	<10	<10	<10

Hair Conditioner

pH: 3.9
% water: 73.7%; A_w: 0.976

Ingredient	%
Phase A	
Deionized Water	q.s. to 100%
Hydroxyethylcellulose	0.30%
Phase B	
Cetrimonium Bromide & Cetearyl Alcohol	1.00%
Stearyl Alcohol	1.00%
Steareth-21	2.50%
Polysorbate 80	0.50%
Lecithin	1.00%
Water	20.00%
Total	100.00%

Test Results

Colony Forming Units per Gram (CFU/g)

Test Organism	Unpreserved Control				Test-Geogard® ECT (1%)			
	Initial Challenge			Rechallenge	Initial Challenge			Rechallenge
	24 hrs	7 days	28 days	28 days	24 hrs	7 days	28 days	28 days
S. aureus	$3.5x10^5$	<10	<10	<10	<10	<10	<10	<10
K. pneumoniae + E. gergoviae	$9.4x10^5$	$3.4x10^5$	$2.6x10^8$	$3.5x10^6$	<10	<10	<10	<10
P. aeruginosa + B. cepacia	$4.9x10^5$	$>10^6$	$3.0x10^8$	<10	$2.0x10^2$	<10	<10	<10
C. albicans	$3.3x10^5$	$3.3x10^6$	$2.7x10^6$	$2.8x10^7$	6.0x10	<10	<10	<10
Mixed molds	$2.1x10^4$	$3.5x10^3$	$1.2x10^3$	$1.4x10^4$	<10	<10	<10	<10

Make-Up Remover

pH: 8.1
% water: 44%; A_w: 0.965

Ingredient	%
Deionized Water	q.s. to 100%
Propylene Glycol	2.00%
Glycerin	2.00%
PEG-8	2.00%
Decyl Glucoside	50.00%
Total	100.00%

Test Results

Colony Forming Units per Gram (CFU/g)

Test Organism	Unpreserved Control				Test-Geogard® ECT (1%)			
	Initial Challenge			Rechallenge	Initial Challenge			Rechallenge
	24 hrs	7 days	28 days	28 days	24 hrs	7 days	28 days	28 days
S. aureus	$1.0x10^2$	<10	<10	<10	<10	<10	<10	<10
K. pneumoniae + E. gergoviae	$5.1x10^6$	$8.0x10^6$	$2.5x10^6$	$8.0x10^5$	<10	<10	<10	<10
P. aeruginosa + B. cepacia	$4.5x10^6$	$6.6x10^6$	$1.5x10^6$	$3.2x10^6$	<10	<10	<10	<10
C. albicans	$4.0x10^2$	<10	<10	<10	<10	<10	<10	<10
Mixed molds	$1.1x10^4$	$2.5x10^4$	$2.0x10^4$	$1.0x10^5$	<10	<10	<10	<10

Water in Oil Emulsion Cream (Lot#: AR12-068)

pH: n/a
% water: 75%; A_w: 0.963

Ingredient	%
Phase A	
Deionized Water	q.s. to 100%
Glycerin	3.00%
Sodium Chloride	1.00%
Phase B	
Cyclomethicone & Dimethicone	10.00%
Cyclopentasiloxane	8.50%
Cyclomethicone & Dimethicone & Petrolatum	2.50%
Total	100.00%

Test Results

Colony Forming Units per Gram (CFU/g)

Test Organism	Unpreserved Control				Test-Geogard® ECT (1%)			
	Initial Challenge			Rechallenge	Initial Challenge			Rechallenge
	24 hrs	7 days	28 days	28 days	24 hrs	7 days	28 days	28 days
S. aureus	$8.6x10^4$	<10	<10	<10	<10	<10	<10	<10
K. pneumoniae + E. gergoviae	$5.6x10^4$	<10	<10	<10	<10	<10	<10	<10
P. aeruginosa + B. cepacia	$3.1x10^4$	$2.9x10^3$	<10	$3.4x10^5$	<10	<10	<10	<10
C. albicans	$4.6x10^4$	$1.3x10^4$	$2.9x10^3$	$5.3x10^4$	<10	<10	<10	<10
Mixed molds	$1.2x10^4$	$9.7x10^3$	$7.0x10^3$	$3.4x10^5$	<10	<10	<10	<10

Lotion (Lot# KKL-1446)

pH: 7.85
% water: 89%; A_w: 0.976

Ingredient	%
Deionized Water	q.s. to 100
Glycerin	2.00%
Cyclomethicone & Dimethicone & Phenyl Trimethicone	2.00%
Cyclopentasiloxane	5.00%
Sodium Acrylate/Sodium Acryloyldimethyl Taurate Copolymer & Hydrogenated Polydecane & Sorbitan Laurate & Trideceth-6	2.00%
Total	100.00%

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Test Results

Colony Forming Units per Gram (CFU/g)

Test Organism	Unpreserved Control				Test-Geogard® ECT (1%)			
	Initial Challenge			Rechallenge	Initial Challenge			Rechallenge
	24 hrs	7 days	28 days	28 days	24 hrs	7 days	28 days	28 days
S. aureus	$1.3x10^6$	$1.6x10^4$	$3.0x10^4$	$8.0x10^3$	7.0x10	<10	<10	<10
K. pneumoniae + E. gergoviae	$1.3x10^6$	$9.5x10^5$	$7.0x10^5$	$2.3x10^3$	2.0x10	<10	<10	<10
P. aeruginosa + B. cepacia	$>10^6$	$8.5x10^6$	$4.3x10^7$	$9.8x10^7$	<10	<10	<10	<10
C. albicans	$1.1x10^5$	$1.0x10^5$	$9.0x10^5$	$1.5x10^5$	$8.7x10^3$	<10	<10	<10
Mixed molds	$2.3x10^6$	$9.0x10^4$	$1.6x10^4$	$7.0x10^4$	$1.8x10^3$	<10	<10	<10

Formulation Recommendations

- Versatile, clear liquid
- Can be easily added directly to most any system
- Compatible with most ingredients used in personal care
- For emulsified systems
  - Can be easily integrated post-emulsification at temperatures below 45°C
  - Limited pH restrictions

Global Regulatory

Europe
- All ingredients approved (Annex V to Regulation EC/1223/2009 formerly Annex VI to Council Directive 76/768/EEC)
  - Max concentration of 1% Benzyl Alcohol, 0.5% Salicylic Acid and 0.6% Sorbic Acid

Japan
- All ingredients approved (JNCI)
  - Max concentration of 1% Benzyl Alcohol, 0.2% Salicylic Acid and 0.6% Sorbic Acid
  - Benzyl Alcohol is not approved as a preservative but can be used as a general cosmetic ingredient

United States
- All ingredients allowed (CIR/PCPC)
  - Max concentration of 1% Benzyl Alcohol, 0.5% Salicylic Acid and 0.6% Sorbic Acid

General
- Cannot be used in products for children under 3 except for shampoo

Typical Properties	
Appearance	Clear, colorless to straw
Color (Gardner)	2 Max.
Odor	Characteristic

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Geogard Ultra by Lonza

Personal Care

Europe

Geogard Ultra™

Next-Generation Preservation

Key Product Benefits:

- Has a wide range of global regulatory acceptance
- Broad spectrum activity
- ECOCERT/COSMOS-accepted , NATRUE-approved and Soil Association-approved
- Wide applicability
- Added moisturization benefit

INCI Name: Gluconolactone & Sodium Benzoate & Calcium Gluconate

Recommended Use Level

0.75–2.0%

Description

Geogard Ultra™ is a synergistic blend comprised of gluconolactone and sodium benzoate. What makes this preservative unique is the synergy between the two ingredients, allowing for its broad spectrum efficacy. Typically, organic acids on their own are too weak and often require a co-preservative or booster in order to perform optimally. The gluconolactone in this blend works together with the sodium benzoate to act as an efficient preservative booster that is also non-GMO. Geogard Ultra™'s gluconolactone works by slowly releasing gluconic acid over time, which helps contribute to the preservation.

Chemical Compound Breakdown	CAS No.	EINECS No.
D-glucono-1,5-lactone	90-80-2	202-016-5
Sodium benzoate	532-32-1	208-534-8
Calcium gluconate	299-28-5	206-075-8

Chemical Compound Breakdown	Percentage
D-glucono-1,5-lactone	70–80%
Sodium benzoate	22–28%
Calcium gluconate	1%

Applications

- Baby care
- Baby wipes
- Body butter
- Body wash
- Conditioner
- Cream
- Deo/anti-perspirant
- Eye creams/gels
- Eye shadow
- Face lotion
- Face wipes
- Facial cream
- Foundation
- Hair gel
- Hand soap
- Lipstick/gloss
- Lotion
- Make up remover
- Oil in Water
- Oral care
- Powder
- Shampoo
- Suncare
- Toner
- Water in Oil

Geogard Ultra™ can be used at 1.0 to 2.0 % as a stand-alone preservative system, but can also be used successfully at lower levels (0.25% to 1.0%) when combined with other synthetic or natural preservatives, preferably good bactericides. Lonza can recommend combinations upon request.

Efficacy

Microbiological Challenge Studies

Studies were run using different concentrations of Geogard Ultra™ in various formulations to see efficacy against various bacteria and fungi. All samples were inoculated at the beginning of the study, sampled at 7, 14 and 28 days.

In these challenge studies, the bacterial pool consisted of *S.aureus*, *P.aeruginosa* and *E.coli*, and the fungal pool of *C.albicans* and *A.brasiliensis*.

Moisturizing Cream

(pH = 5.28)

Ingredient	%W/W
Water, deionized	q.s
Caprylic Triglyceride	20.00%
Sorbitan Monostearate	2.00%
PEG Stearate	1.50%
Glyceryl Stearate	2.00%
Decaglyceryl Decaoleate	5.00%
UV absorber	optional
Thickener	optional
Preservative	1.5% Geogard Ultra™
Total:	100.00%

Bacterial Counts (CFU/gram)

Sample#	TestSamples	Day0	Day7	Day14	Day28
1	Unpreserved Moisturizer	$9.5x10^6$	$4.2x10^5$	$8.9x10^4$	<10
2	Moisturizer with 1.5% Geogard Ultra™	$6.5x10^6$	<10	<10	<10

Fungal Counts (CFU/gram)

Sample#	Test Samples	Day 0	Day 7	Day 14	Day 28
3	Unpreserved Moisturizer	$8.8x10^5$	$1.7x10^5$	$1.9x10^5$	$2.8x10^5$
4	Moisturizer with 1.5% Geogard Ultra™	$2.1x10^5$	<10	<10	<10

Anionic Protein Shampoo
(pH = 5.42)

Ingredient	%W/W
Water, deionized	q.s
Sodium Lauryl Ether Sulfate	15.0%
Triethanolamine Lauryl Sulfate	10.0%
Cocamide DEA	3.0%
Anhydrous Protein	1.0%
50% Aqueous Citric acid	pH adjuster
Preservative	1.5% Geogard Ultra™
Total	100.00%

Bacterial Counts (CFU/gram)

Sample#	Test Samples	Day 0	Day 7	Day 14	Day 28
1	Unpreserved Shampoo	$9.5x10^6$	$4.76x10^7$	$1.06x10^8$	$2.0x10^7$
2	Shampoo with 1.5% Geogard Ultra™	$5.2x10^5$	<10	<10	<10

Fungal Counts (CFU/gram)

Sample#	Test Samples	Day 0	Day 7	Day 14	Day 28
3	Unpreserved Shampoo	$6.6x10^5$	$2.0x10^5$	$3.0x10^5$	$1.7x10^7$
4	Shampoo with 1.5% Geogard Ultra™	$4.4x10^5$	<10	<10	<10

Hair Conditioner
(pH = 4.89)

Ingredient	% W/W
Water, deionized	q.s
Polysorbate 80 (Glycosperse® 0-20)	0.5%
Lecithin	1.0%
Distearyldimonium Chloride (Varisoft TA100)	2.0%
Cetyl alcohol	2.1%
Cetearyl alcohol	1.5%
POE 4 Lauryl Alcohol (Ethosperse® LA-4)	3.1%
10% Aqueous Sodium Hydroxide	pH adjuster
Preservative	1.0% Geogard Ultra™
Total:	100.00%

Bacterial Counts (CFU/gram)

Sample#	Test Samples	Day 0	Day 7	Day 14	Day 28
1	Unpreserved Conditioner	8.3×10^6	4.8×10^7	2.4×10^6	9.0×10^6
2	Conditioner w/ 1.0% Geogard Ultra™	3.5×10^5	< 10	< 10	< 10

Fungal Counts (CFU/gram)

Sample#	Test Samples	Day 0	Day 7	Day 14	Day 28
3	Unpreserved Conditioner	4.2×10^6	1.8×10^7	8.3×10^5	3.7×10^5
4	Conditioner w/ 1.0% Geogard Ultra™	4.1×10^4	2.0×10^2	<10	<10

Wet Wipe Liquor
(pH = 5.54)

Ingredient	%W/W
Water	q.s to 100
Decyl glucoside (Plantaren® 2000)	0.25%
Polysorbate 20 (Glycosperse® L-20)	0.30%
Disodium EDTA	0.20%
Sodium citrate	3.00%
Geogard Ultra™	2.00%
Total	100.00%

(pH adjustments for in-situ buffer)

Bacterial Counts (CFU/gram)

Sample#	Test Samples	Day 0	Day 7	Day 14	Day 21	Day 28
1	SPC nonwoven (unpreserved)	1.6×10^6	3.1×10^5	$>3.9 \times 10^6$	$>3.9 \times 10^6$	$>3.9 \times 10^6$
2	SPC nonwoven with 2% Geogard Ultra™	2.1×10^6	<100	<100	<100	<100
3	Spunlace nonwoven (unpreserved)	2.6×10^6	3.0×10^6	$>3.9 \times 10^6$	$>3.9 \times 10^6$	$>3.9 \times 10^6$
4	Spunlace nonwoven with 2% Geogard Ultra™	1.9×10^6	<100	<100	<100	<100

Fungal Counts (CFU/gram)

Sample#	Test Samples	Day 0	Day 7	Day 14	Day 21	Day 28
5	SPC nonwoven (unpreserved)	7.7×10^4	2.4×10^6	6.4×10^6	4.1×10^5	1.2×10^6
6	SPC nonwoven with 2% Geogard Ultra™	7.8×10^4	1.0×10^2	<100	<100	<100
7	Spunlace nonwoven (unpreserved)	1.2×10^5	5.5×10^5	8.8×10^5	1.1×10^6	1.2×10^6
8	Spunlace nonwoven with 2% Geogard Ultra™	9.5×10^4	<100	<100	<100	<100

There is also a moisturization benefit on the skin with the Geogard Ultra™. In the same moisturizing cream formulation used to demonstrate preservative efficacy, Geogard Ultra™ produced a quantitative moisturization benefit to the skin. Over a period of time, Geogard Ultra™ produced a moisturizing effect that was superior to the use of 2 % glycerin.

Average Moisturizing Effect on 9 Subjects Over Five Days

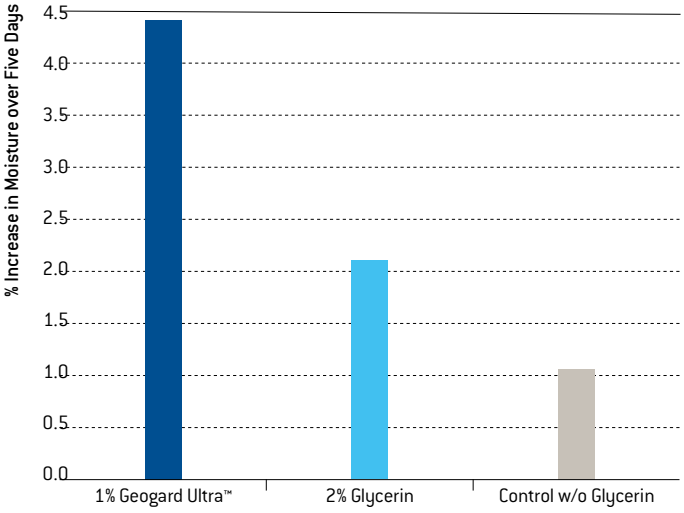

Fig. 1

Global Regulatory

Europe
- Max concentration of sodium benzoate is based on benzoic acid content
- Max concentration of benzoic acid is 2.5% for rinse-off
- Max concentration of benzoic acid is 0.5% for leave-on

Japan
- 1.0% total max level of sodium benzoate

US
- 5.0% total max level of sodium benzoate

General
- Compliance with ECOCERT/COSMOS and Soil Association

Formulation Recommendations

- Water soluble
- Compatible with a wide variety of formulation ingredients as well as most types of cationic, nonionic and anionic systems
- Can be used effectively over a pH range of 3 to 6 and can be added at both room and elevated temperatures
- Soluble up to 4% in ambient water; it can be easily dispersed in glycols and alkyl sulfates
- To maximise the pH stability of the final formulation, it may be necessary to employ use of a sodium citrate buffer and pH adjustment as described below...
  1. Dose the final product with the required level of Geogard Ultra™ along with a 1.5x amount of sodium citrate. So, a 2% dose of Geogard Ultra™ should be accompanied by 3% sodium citrate
  2. Mix thoroughly to ensure all solids have dissolved and adjust the pH of the formulation to 7.00 - 7.25 with 30% sodium hydroxide
  3. Finally, adjust the pH to desired final product pH (pH 5.4 – 5.5 is ideal) with dilute sodium hydroxide or citric acid solution

Solubility Data

Solvent	Soluble/Insoluble
Water	Soluble
Propylene Glycol	Dispersible
Glycerin	Soluble
Ethanol	Insoluble
Mineral Oil	Dispersible
Vegetable Oil	Insoluble
Silicone (Dimethicone)	Insoluble
Alkyl Sulfates	Dispersible

Typical Properties	
Gluconolactone,%	70% Minimum
Sodium Benzoate,%	22% Minimum
Appearance	Free flowing, white powder
Activity	99%

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Iscaguard PFA by ISCA

Iscaguard® PFA

Iscaguard® PFA is a "preservative free additive" with a synergistic combination of multifunctional cosmetic raw materials with broad-spectrum antimicrobial protection. Iscaguard® PFA may be classed as a "preservative free system". This system represent an alternative to traditional cosmetic preservatives allowing self-preserving formulations thereby reducing irritant and sensitizing potentials.

Iscaguard® PFA is stable and active over a pH range of 4 to 8. Typical use levels for Iscaguard® PFA range from 0.5% to 1.5%. It is synergistic in combination with chelating agents.

Regulatory Status

Iscaguard® PFA is permitted worldwide for use in both leave-on and rinse-off personal care products.

EU – allowed without restriction in all products (not listed on Annex VI)
USA – allowed without restriction in all products
Japan – allowed without restrictions in all products

Phenethyl alcohol is judged safe for use in cosmetics to 1.0%. Based on the CIR review for phenethyl alcohol and the good toxicity assessment for caprylyl glycol Iscaguard® PFA should be safe in cosmetics up to 1.8%

INCI name: phenethyl alcohol, caprylyl glycol

Using Iscaguard® PFA

Iscaguard® PFA is particularly suitable for emulsions, oil and surfactant based formulations and may be used in aqueous formulations upto its solubility limit i.e. 0.6%. Iscaguard® PFA can be added to formulation at temperatures up to 80°C, prolonged heating at elevated temperatures is not recommended.

Physical/Chemical Characteristics

Caprylyl glycol	35 – 45%
Phenethyl alcohol	55 – 65%

Appearance	Clear colorless liquid
Odor	Mild rose-like odor
Density	0.98 g/ml
pH (0.5% in water)	4.6

Table 35

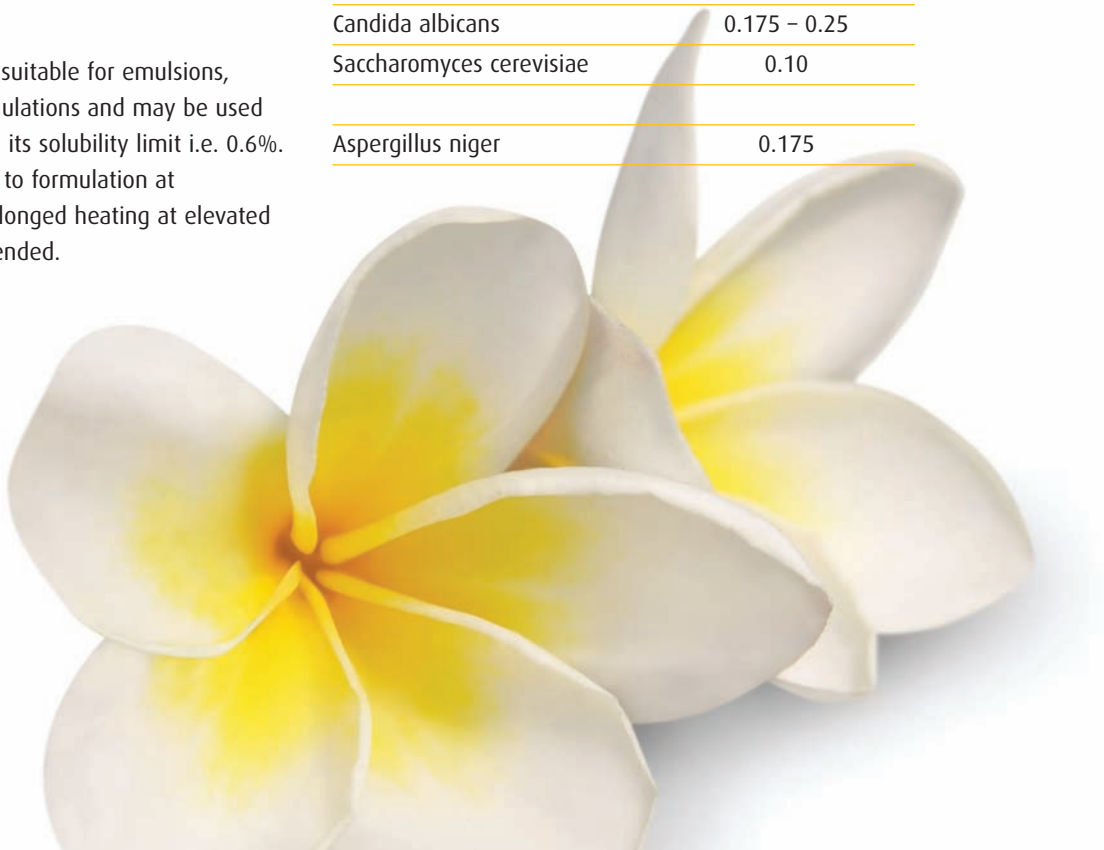
Solubility of Iscaguard® PFA (% w/w @ 25°C)

Water	0.6%
Ethanol	>50%
Propylene glycol	>50%
Butylene glycol	>50%

Table 36

Antimicrobial activity of Iscaguard® PFA

Microorganism	Minimum inhibitory concentration(%)
Pseudomonas aeruginosa	0.10 - 0.30
Escherichia coli	0.10 – 0.20
Proteus vulgaris	
Staphylococcus aureus	0.175 – 0.2
Bacillus subtilis	0.20
Enterococcus hirae	0.20
Candida albicans	0.175 – 0.25
Saccharomyces cerevisiae	0.10
Aspergillus niger	0.175



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Technical Service

ISCA support their product range with a telephone advisory service and in-house microbiological testing facilities. Please contact any member of our team for further details.

Disclaimer

Whilst the information contained herein is accurate to the best of our knowledge, no warranty is either expressed or implied. It is the responsibility of the individual to ensure that their products will remain preserved over the anticipated shelf life.

United Registrar of Systems Cert No.11860

Lexgard Natural by Inolex

Product Bulletin

Lexgard® Natural

INCI ADOPTED NAME

Glyceryl Caprylate (and) Glyceryl Undecylenate

GENERAL INFORMATION

Lexgard Natural is an all-natural multi-functional ingredient system for preservative-free and self-preserving cosmetics.

Lexgard Natural has the following features:

- 100% vegetable derived
- No petrochemical content
- Ecocertified by the leading natural cosmetic standards
- Its eco-credentials are far superior to “nature identical” petrochemical ingredients

Lexgard Natural is composed of high purity monoesters of caprylic acid (C8 acid) and undecylenic acid (C11 acid). The former is well established for its biostatic activity against bacteria and yeast. The latter is known for its activity against fungus. Many formulations containing **Lexgard Natural** pass challenge tests required in cosmetics.

PRINCIPAL USES

Lexgard Natural is an emollient, co-emulsifier, skin re-fatting agent, and biostatic system. It is especially recommended for use in w/o or o/w emulsion systems such as skin care creams and lotions. It may be incorporated in the oil or water phase at any point during the emulsification process. For antimicrobial performance, a dosage of 1.0% – 1.5% is recommended. **Lexgard Natural** is stable and effective at pH 4.0 - 7.5, with optimal results at pH 5.5 or lower.

PHYSICAL PROPERTIES (TYPICAL)

Appearance.....Liquid to white, solid
Odor ..... Mild, characteristic

STORAGE AND HANDLING

It is recommended that normal safety precautions be employed when handling **Lexgard Natural**. Refer to the material Safety Data Sheet (SDS) for further information.

SAFETY DATA

Refer to the material Safety Data Sheet (SDS) for further information.

STANDARD PACKAGING

Plastic pail, 55 lb (24.9 kg) net weight.

2101 S. Swanson St. | Philadelphia, PA 19148 USA | toll free + 1 800 521 9891 | office + 215 271 0800 | fax + 215 271 6282 | www.inolex.com

Sorbic Acid and Potassium Sorbate as Cosmetic Preservatives
by Eastman

Eastman

Sorbic Acid and Potassium Sorbate as Cosmetic Preservatives

eastman

Contents

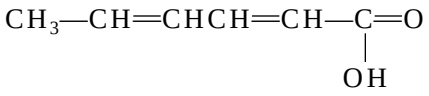
Key Characteristics

- Wide-spectrum antimicrobial
- Good water-to-oil partition coefficient
- Compatible with other cosmetic ingredients
- Effective over a wide pH range
- Nontoxic, safe for human use
- Environmentally safe

Wide-Spectrum Antimicrobials for Maintaining Freshness

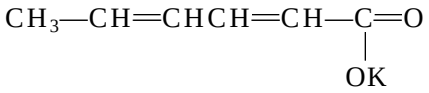
Sorbic acid and potassium sorbate are excellent, safe preservatives for cosmetics and personal care products with a pH lower than 6.5. They have good skin compatibility and are easy to use, especially potassium sorbate in salt form.

Sorbic acid, a straight-chained monocarboxylic acid whose chemical formula is $C_6H_8O_2$, has the following structure:



2,4-Hexadienoic Acid
Sorbic Acid
CAS No. 110-44-1

The structure for the potassium salt known as potassium sorbate ($C_6H_7O_2K$) is:



2,4-Hexadienoic Acid
Potassium Salt
CAS No. 24634-61-5

Sorbic acid was first isolated from the pressed unripened berries of the rowan or mountain ash tree by A. W. Hoffmann, a German chemist, in 1859.

The antimicrobial preservative power of sorbic acid wasn't discovered until 1939–1940. After that, the effectiveness of sorbic acid as a preservative and its physiological safety were thoroughly studied. As early as 1955, both sorbic acid and potassium sorbate were proven to be safe and innocuous. Since that time, sorbates have been approved for use as food preservatives in nearly all countries of the world. Sorbic acid has been used as a preservative in cosmetics since the early 1960s.

Eastman is the only American manufacturer of sorbic acid. Both sorbic acid and its potassium salt are manufactured at a modern plant located at Chocolate Bayou near Alvin, Texas. They are manufactured under rabbinical supervision and are kosher.

The following pages provide a variety of technical data to help determine whether sorbates are suitable for your particular application. The sections give property and solubility information, specific organisms inhibited by sorbates, effectiveness of sorbates under various conditions and use levels, and product safety and regulatory information. Additional information can be obtained by contacting Eastman Chemical Company Technical Service.

Properties

Properties[a]

	Eastman **Sorbic Acid**	***Eastman*** **Potassium Sorbate**
INCI/CTFA Name[b]	Sorbic Acid	Potassium Sorbate
Molecular Weight	112.13	150.22
Water Solubility @ 20°C	0.15%	58.2%
Solubility in Organic Compounds, % by wt @ 20°C		
Ethanol, 100%	12.9	2.0
95%	12.6	6.5
50%	4.8	45.3
20%	0.29	54.6
5%	0.16	57.4
Ethyl Ether	5.0	0.1
Fatty Oils	0.6–1.2	<0.1
Propylene Glycol	5.5	20
Glycerol	0.31	0.20
Acetic Acid, Glacial	11.5	—
Acetone	9.2	0.1
Vapor Pressure, mm Hg		
@ 20°C	<0.001	NA
120°C	10	NA
140°C	43	NA
Flash Point, °C (°F) (COC, ASTM D 92)	127 (260)	none
Ionization Constant @ 25°C	1.73×10^{-5}	—
Assay, Dry Basis	99.0%–101.0%	98.0%–101.0%
Identification	Passes Food Chemicals Codex Specifications	
Appearance	White to off-white, free flowing	
Melting Range	132.0°–135.0°C	Decomposes above 270°C
Water Content	0.5% maximum	1.0% maximum
Alkalinity/Acidity	—	1.1 mL 0.1N NaOH to 0.8 mL 0.1N HCl per 1.1 g
Products Available	Powder, dust-free	Powder or granular

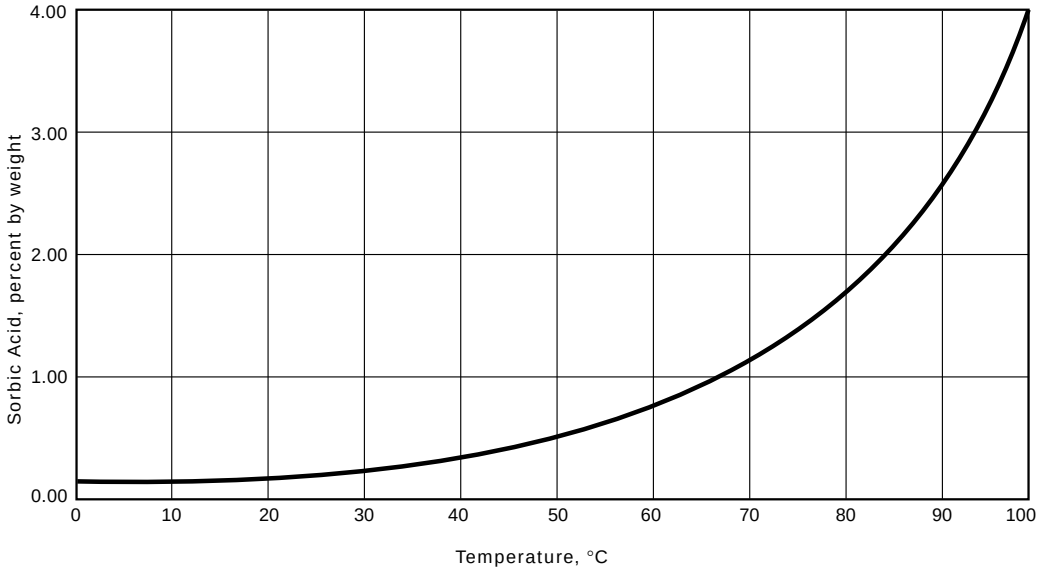
[a]*Properties reported here are typical of average lots. Eastman makes no representation that the material in any shipment will conform to the values given.*
[b]*International Nomenclature Cosmetic Ingredient; Cosmetic, Toiletry, and Fragrance Association.*

NA—*Not Applicable*

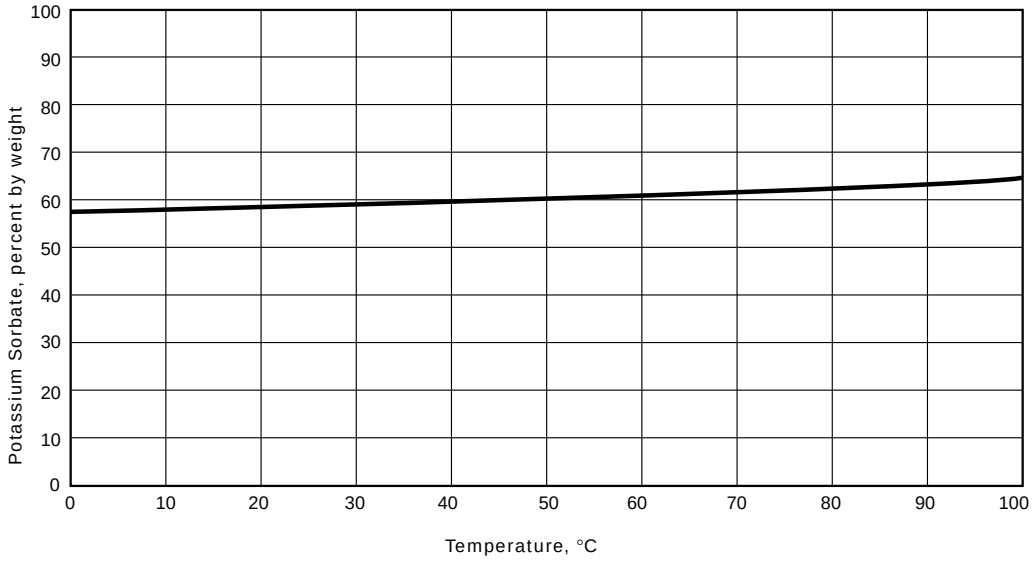
Eastman sorbic acid and *Eastman* potassium sorbate are highly refined, white to off-white, free-flowing powders or granules. Sorbic acid provides greater antimicrobial potency than potassium sorbate. However, in water, sorbic acid is barely soluble while potassium sorbate is extremely soluble. Therefore, potassium sorbate is usually chosen as a preservative for cosmetic products. The potency of the salt on an equivalent weight basis to the acid is 74%. Thus, for equal preservative power, four parts of potassium salt must be used to equal three parts sorbic acid.

Solubility in Water

SORBIC ACID, 0° TO 100°C

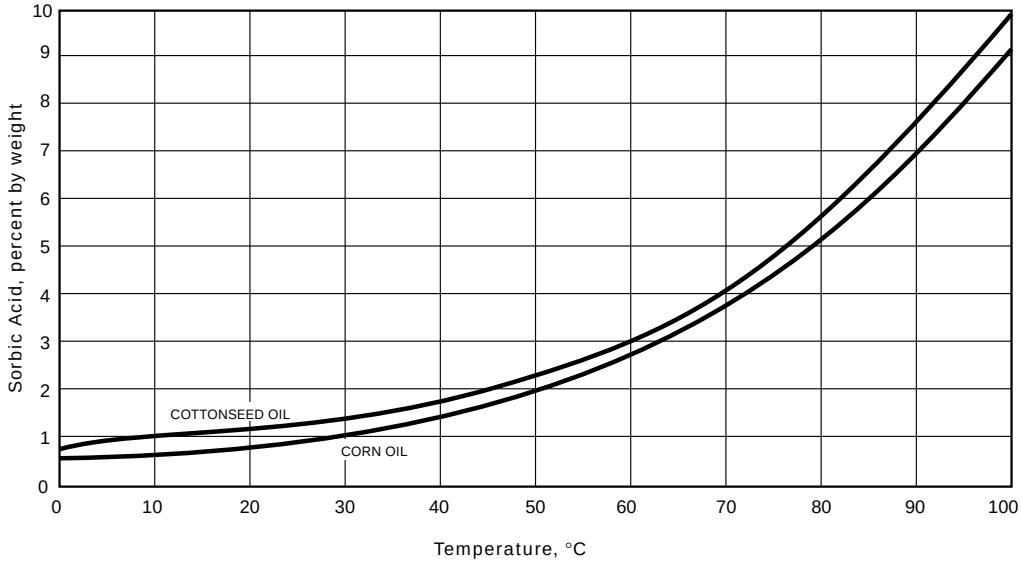


POTASSIUM SORBATE, 0° TO 100°C

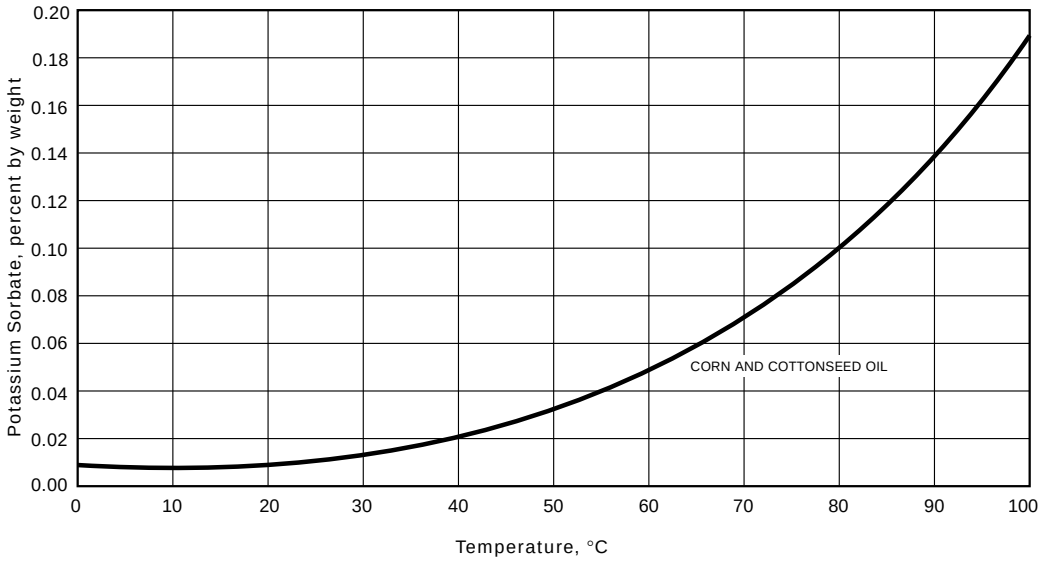


Solubility in Corn and Cottonseed Oils

SORBIC ACID, 0° TO 100°C

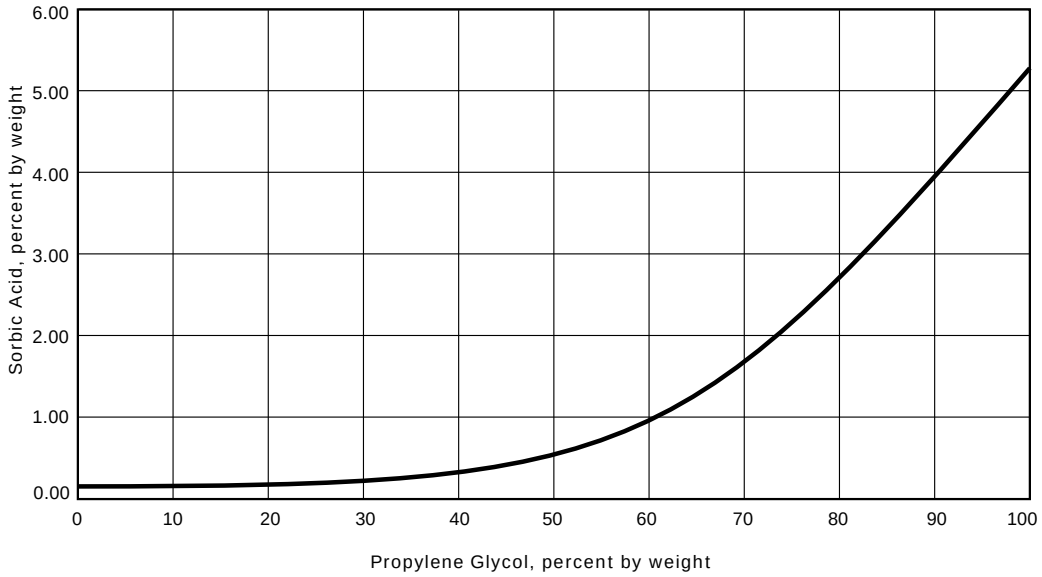


POTASSIUM SORBATE, 0° TO 100°C

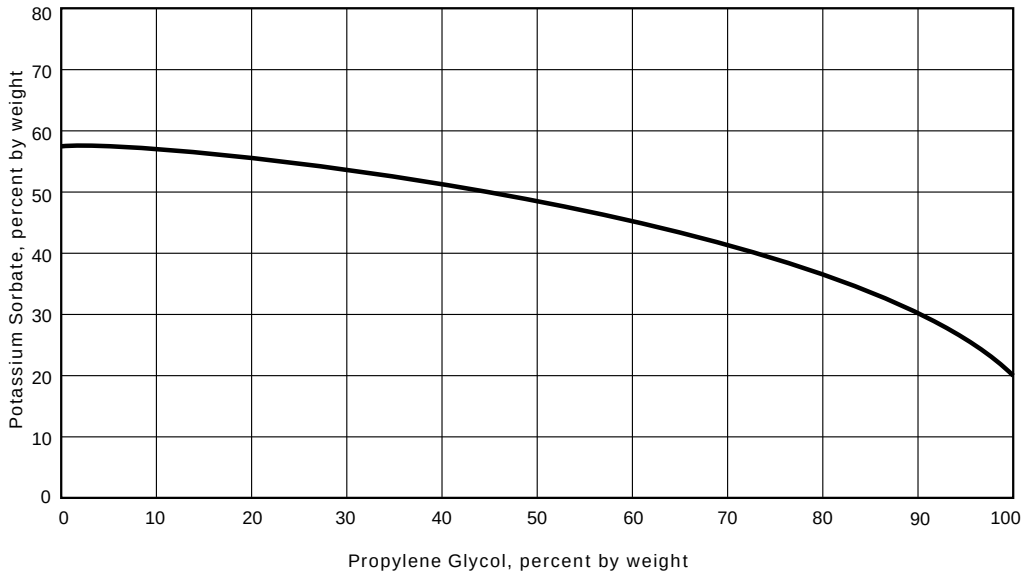


Solubility in Propylene Glycol/ Water Solutions

SORBIC ACID, 20°C



POTASSIUM SORBATE, 20°C



Above about 60°C (140°F), sorbic acid begins to sublime. This volatility should be considered when sorbate is to be added prior to a heating step in the existing process.

Sorbates have a relatively high water-to-oil partition coefficient. A high water-to-oil partition coefficient means a high concentration of sorbates in the aqueous phase and a low concentration in the oil phase. As the pH of the formulation increases (approaching pH = 7) and sorbic acid converts to the salt form, the partition coefficient increases. A high partition coefficient is favorable because microorganisms reproduce in the aqueous phase and, in the case of an emulsion, at the interface between the aqueous and oil phase. Therefore, a balance is achieved. Even though the potassium sorbate has less antimicrobial potency than sorbic acid, it offers better solubility in water where antimicrobial effectiveness is most needed.

Sorbates are compatible with other cosmetic ingredients. Unlike the p-hydroxybenzoic acid esters (parabens), sorbic acid remains active when used with nonionic emulsifiers. Sorbates do have an antagonistic effect on chlorhexidin digluconate, which is inactivated by the potassium ion. However, chlorhexidin digluconate and sorbates are not normally used in the same products. Sorbates are used in leave-on or rinse-off products and chlorhexidin digluconate is used in oral hygiene products. Several other cosmetic preservatives are also antagonistic to chlorhexidin digluconate.

Under certain conditions, sorbic acid may oxidize and cause slight color changes in the cosmetic product. This can normally be prevented by adding 0.1%–0.3% citric acid to the product. Citric acid may already be added to cosmetics to obtain a skin-neutral pH. Highly concentrated solutions of sorbic acid and potassium sorbate may oxidize and become discolored during prolonged storage, especially when exposed to sunlight. Therefore, sorbate stock solutions should be used up as soon as possible.

Antimicrobial Effectiveness

Most cosmetics have great potential for microbial contamination and growth, especially creams and lotions that are packed in jars, opened frequently, and applied to the skin with the fingers. Brushes that are used to apply makeup around the eyes or other parts of the face touch the skin and the cosmetic repeatedly. Each use increases the chance for contamination. Several cases of eye ulceration and partial or complete blindness have been attributed to mascaras contaminated with pseudomonas. Cosmetic contamination may also occur because consumers leave the containers open for a period of time. Moreover, most cosmetics are stored at room temperature and the warm temperatures stimulate the growth of microorganisms. In addition, the ingredients in cosmetics contain all the things microorganisms like—water, oils, peptides, and a variety of carbohydrates.

All of these factors mean that good preservatives are essential for cosmetics. In fact, cosmetics need better preservation than foods normally stored in cooler temperatures and consumed quickly. Cosmetic preservatives must be strong, but they must also be nonirritating to skin. Sorbates fit both of these criteria.

Sorbic acid is effective against small populations of common microorganisms in cosmetics. Cosmetic preservatives are not intended to combat extremely high counts of bacteria. They are intended to control small populations that would normally be present in products manufactured under clean, sanitary conditions. Sorbic acid can be metabolized by some species of organisms when they are present in extremely high concentrations. However, this situation should not occur when good manufacturing practices are employed.

When selecting a preservative and establishing a use level, two factors are particularly important: the type of microorganisms that can potentially grow and the pH of the product. Other factors to consider include water content, storage temperature, shelf life expectancy, and potential for abuse in distribution and use. Generally higher sorbate levels are required when the water content is higher and storage temperatures are warmer.

Factors That Influence the Effectiveness of Preservatives

Initial Contamination Level

- Raw materials
- Water supply
- Processing sanitation—equipment and premises

Composition of Cosmetic / Personal Care Product

- pH of the product
- Water content
- Antimicrobial effects of other ingredients

Distribution and Use

- Packaging
- Storage temperature
- Shelf life expectancy
- Potential for contamination by consumer

Microorganisms Inhibited by Sorbates

The following charts list the most common microorganisms inhibited by sorbates. These organisms are not necessarily found in cosmetics.

Molds

Alternaria citri[a]
Alternaria tenuis[b]
Alternaria spp.[c]
Ascochyta cucumis[b]
Ascochyta sp.[b]
Aspergillus clavatus[a]
Aspergillus elegans[b]
Aspergillus flavus[b]
Aspergillus fumigatus[b]
Aspergillus glaucus[c]
Aspergillus niger[b,c]
Aspergillus ochraceus[a]
Aspergillus parasiticus[a]
Aspergillus sydowi[b]
Aspergillus terreus[b]
Aspergillus unguis[b]
Aspergillus versicolor[b]
Botrytis cinerea[a]
Cephalosporium sp.[b]
Cercospora sp.[b]
Chaetomium globosum[b]
Cladosporium cladosporiodes[b]
Colletotrichum lagenarium[b]
Cunninghamella echinulata[b]
Curvularia trifolii[b]
Fusarium episphaeria[b]
Fusarium moniliforme[b,c]
Fusarium oxysporum[b,c]
Fusarium roseum[c]
Fusarium rubrum[a]
Fusarium solani[b,c]
Fusarium tricinctum[a]
Geotrichum candidum[a]
Geotrichum sp.[b] (2 strains tested)
Gliocladium roseum[b]
Helminthosporium sp.[b] (2 strains tested)
Heterosporium terrestre[b]
Humicola fusco-atra.[b]
Mucor silvaticus[b]
Mucor spp.[b,c] (5 strains tested)
Myrothecium roridum[b]
Myrothecium verrucaria[b]
Myrothecium sp.[b]
Papularia arundinis[b]
Penicillium atromentosum[b]
Penicillium chermesinum[b]
Penicillium chrysogenum[c]
Penicillium citrinum[a]
Penicillium digitatum[a]
Penicillium duclauxi[b]
Penicillium expansum[b]
Penicillium frequentans[b]
Penicillium funiculosum[b]
Penicillium gladioli[b]
Penicillium herquei[b]
Penicillium implicatum[b]
Penicillium italicum[a]
Penicillium janthinellum[b]
Penicillium notatum[c]
Penicillium oxalicum[b,c]
Penicillium patulum
Penicillium piscarium[b]
Penicillium purpurogenum[a]
Penicillium restrictum[b]
Penicillium roquefortii[c]
Penicillium rugulosum[b]
Penicillium sublateritium[b]
Penicillium thomii[b]
Penicillium urticae[b]
Penicillium variabile[b]
Penicillium spp.[b,c] (2 strains tested)
Pestolotiopsis macrotricha sp.[b]
Phoma sp.[b]
Pullularia pullulans[b,c]
Rhizoctonia solani[a]
Rhizopus arrhizus[b]
Rhizopus nigricans[b,c]
Rosellinia sp.[b]
Sporotrichum pruinosum[b]
Stagonospora sp.[b]
Stysanus sp.[b]
Thielavia basicola[b]
Trichoderma viride[b]
Truncatella sp.[b]

[a]*Eastman Chemical Company unpublished data.*
[b]*Bell, T. A., Etchells, J. L., and Borg, A. F.,* J. Bacteriology *77 573 (1959).*
[c]*York, G. K., Dissertation, University of California Davis (1960).*

Yeasts

Brettanomyces clausenii[c]
Brettanomyces versatilis[b]
Candida albicans[b,c]
Candida krusei[b,c]
Candida tropicalis[c]
Candida mycoderma[c]
Cryptococcus terreus[c]
Cryptococcus neoformans[b]
Cryptococcus sp.[c]
Debaryomyces membranaefaciens[c]
Debaryomyces membranaefaciens var. hollandicus[b]
Debaryomyces spp.[c]
Endomycopsis ohmeri[b]
Hansenula anomala[c]
Hansenula saturnus[c]
Hansenula subpelliculosa[b,c]
Oospora sp.[c]
Pichia alcoholophila[b]
Pichia membranaefaciens[c]
Pichia polymorpha[c]
Pichia silvestris[c]
Pichia sp.[b]
Rhodotorula flava[b]
Rhodotorula glutinis[b]
Rhodotorula rubra[b,c]
Rhodotorula spp.[b]
Saccharomyces cerevisiae[b,c]
Saccharomyces cerevisiae var. ellipsoideus[c]
Saccharomyces carlsbergensis
Saccharomyces fragilis[b,c]
Saccharomyces rouxii[c]
Saccharomyces delbrueckii[b]
Saccharomyces lactis[b]
Schizosaccharomyces octosporus[c]
Sporobolomyces sp.[c]
Torulaspora rosei[b,c]
Torulopsis candida[b]
Torulopsis caroliniana[b]
Torulopsis minor[b]
Torulopsis polcherrima[c]
Torulopsis versitalis lipofera[b]
Zygosaccharomyces globiformis[b]
Zygosaccharomyces halomembranis[b]

Bacteria

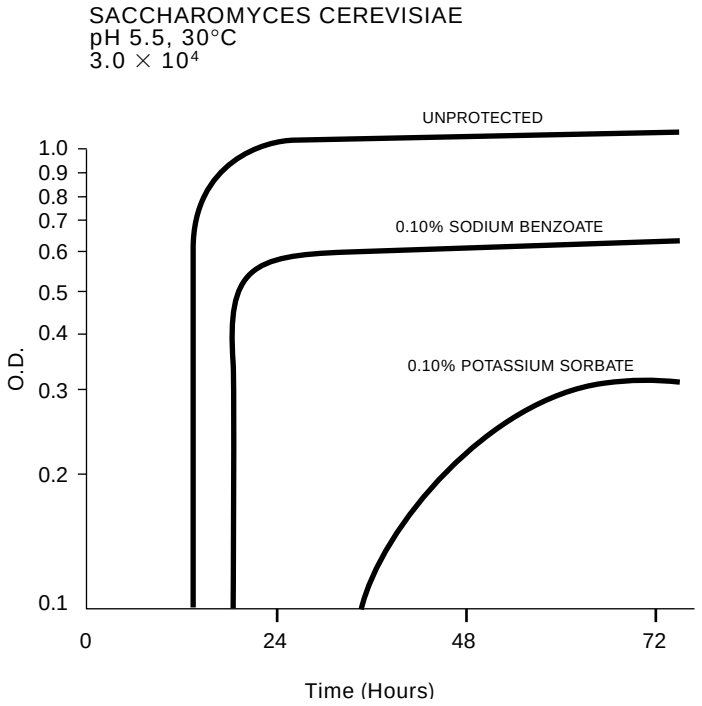
Acetobacter aceti[c]
Acetobacter xylinum[c]
Achromobacter sp.[c]
Alcaligenes faecalis[c]
Azotobacter agilis[c]
Bacillus coagulans[c]
Bacillus cereus[c]
Bacillus poymyxa[c]
Bacillus stearothermophilus[c]
Bacillus subtilis[c]
Clostridium perfringens[a]
Clostridium sporogenes[a]
Clostridium tetani[d]
Enterobacter aerogenes[c]
Escherichia coli[c]
Escherichia freundii[c]
Klebsiella species[d]
Lactobacillus brevis[a]
Micrococcus sp.[c]
Propionibacterium zeae[c]
Propionibacterium freundenreichii
Proteus vulgaris[c]
Pseudomonas aeruginosa[d]
Pseudomonas fragi[c]
Pseudomonas fluorescens[a]
Pseudomonas sp.[c]
Salmonella heidelberg[a]
Salmonella montevideo[a]
Salmonella typhimurium[c]
Salmonella enteritidis[c]
Sarcina lutea[c]
Serratia marcescens[c]
Staphylococcus aureus[c]
Streptococcus pyogenes[d]
Vibrio parahaemolyticus[a]

[a]*Eastman Chemical Company unpublished data.*
[b]*Bell, T. A., Etchells, J. L., and Borg, A. F.,* J. Bacteriology *77 573 (1959).*
[c]*York, G. K., Dissertation, University of California Davis (1960).*
[d]*Jager, M., Preservatech Conference Proceedings, pp 39–50 (1995).*

Relationship of pH to Antimicrobial Effectiveness

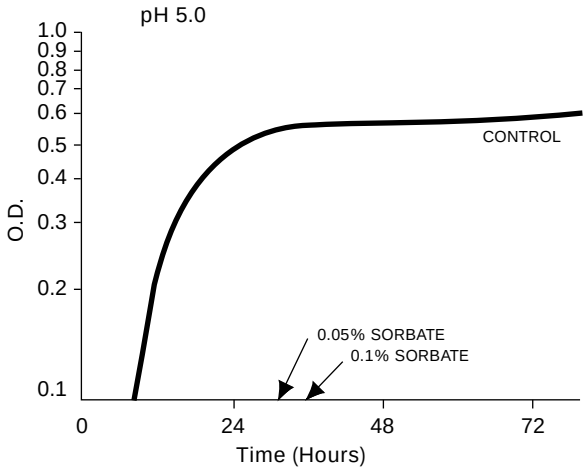
The antimicrobial potency of all commercial cosmetic preservatives is pH-dependent. Sorbates are more effective at higher pH ranges than other organic acids used as preservatives. Sorbates are effective up to 6.5, whereas benzoates are effective to only 4.5. These preservative compounds can be used in either the acid or salt form. Their antimicrobial activity is mainly due to the undissociated acid molecule. Sorbates are most effective when used below pH 6.0. They function up to pH 6.5, but are relatively ineffective above pH 7.0.

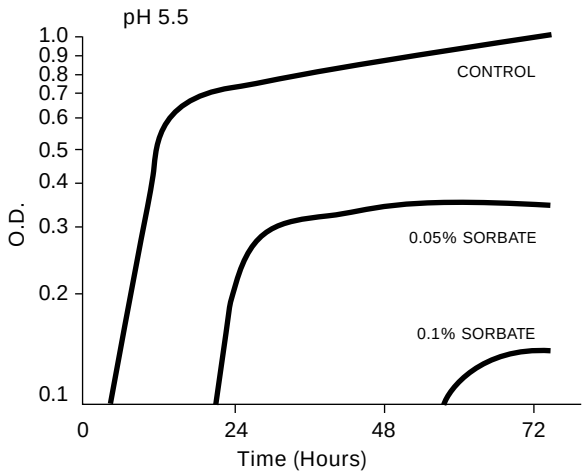
The graph shows the relative inhibition of yeast by equal concentrations of sorbate and benzoate at pH 5.5 and 30°C when a broth is inoculated with 3×10^4 organisms / mL. Growth is measured by the optical density of the broth. Sorbate significantly delays growth, and the amount of ultimate growth at 72 hours is far less.

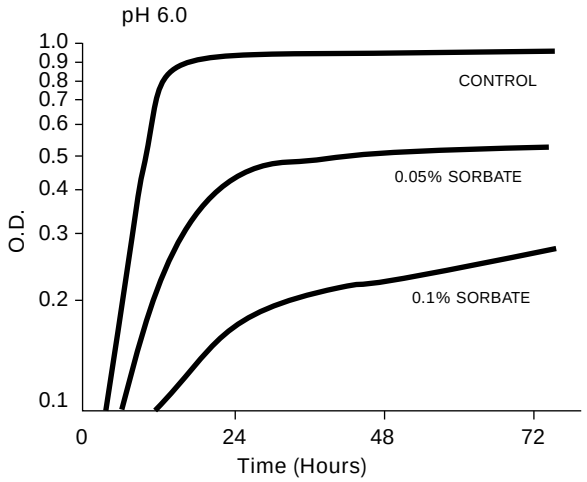


The following graphs show the effectiveness of sorbate at pH 5.0, 5.5, 6.0, and 6.5

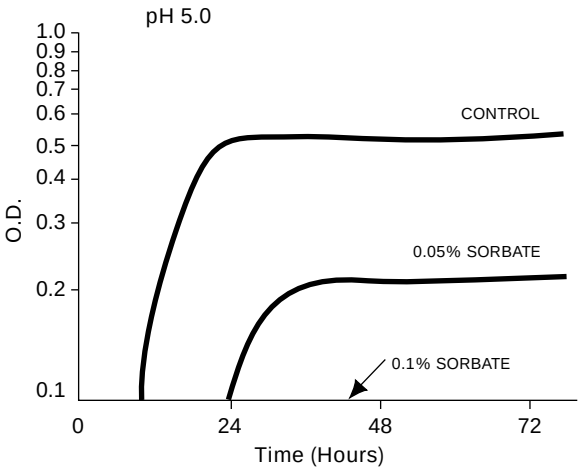
ESCHERICHIA COLI

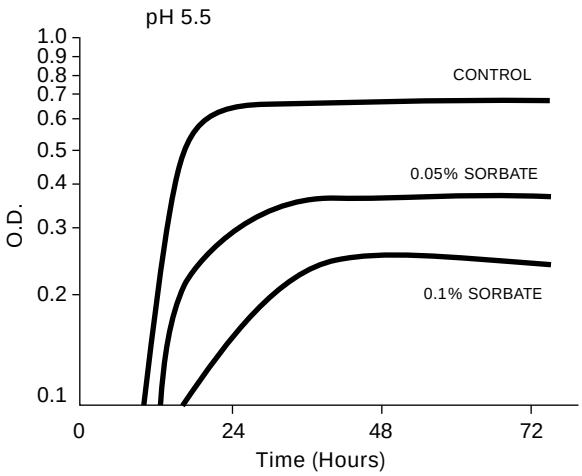


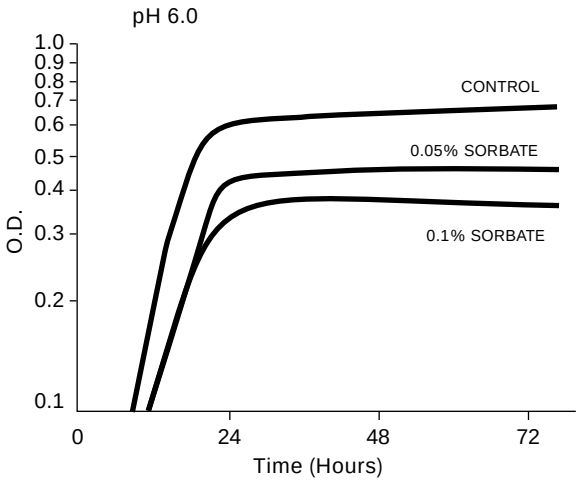




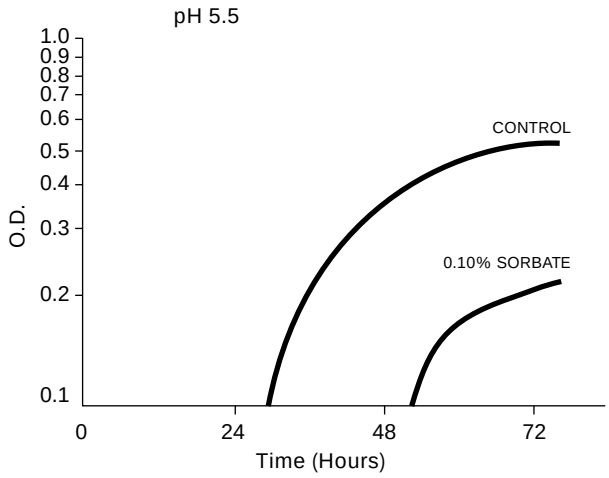
SACCHAROMYCES CEREVISIAE

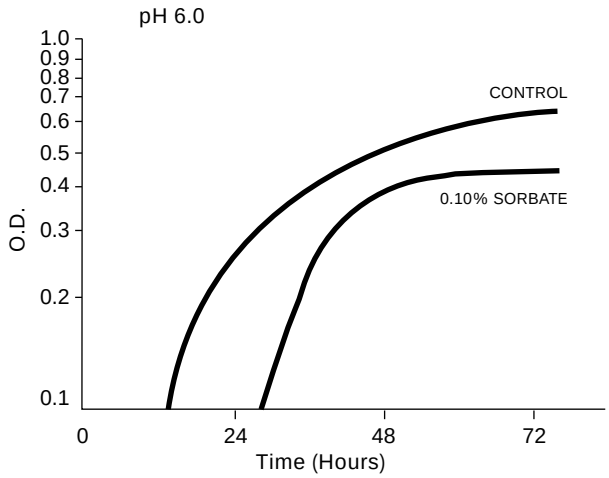


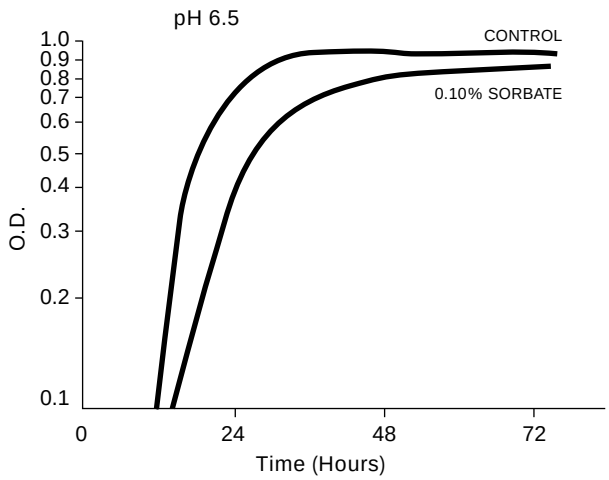




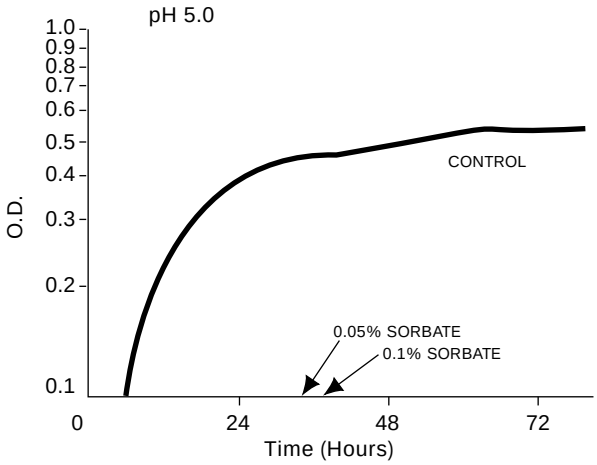
STAPHYLOCOCCUS AUREUS

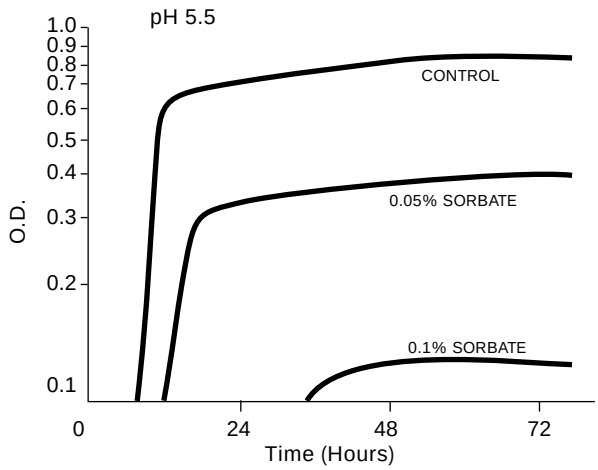


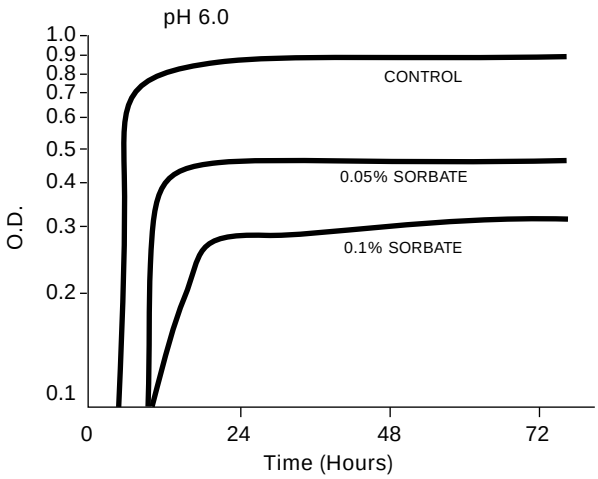




SALMONELLA







Sorbate Use Levels

Normally, *Eastman* sorbic acid and *Eastman* potassium sorbate are effective in a concentration range of 0.05% to 0.3% by weight. Generally, the higher the sorbate level, the longer the microbial growth will be inhibited. Increasing the potential of exposure to microbial contamination (e.g., cosmetic containers that are opened frequently, contents that last beyond a single use, or a product that is particularly susceptible to attack) requires the use of a higher level of preservative.

In a study done on a rinse-off product, potassium sorbate was very effective in combating microorganisms. The product was inoculated with Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Aspergillus niger, and Candida albicans. When the rinse-off product (pH 5.5) contained 0.4% potassium sorbate, fewer than 10 microorganisms remained in the product after both one week and one month even though the initial concentration had been as high as 6.5×10^5. For most of the microorganisms tested, 0.4% potassium sorbate in combination with 0.1% citric acid reduced the microorganism counts faster than potassium sorbate alone.

Another study showed that 0.05% to 0.2% sorbates are required to combat gram positive bacteria such as Staphylococcus aureus, Streptococcus pyrogenes, and Clostridium perfringens. Greater than 0.4% sorbates are required to fight Clostridium tetani.

It also showed that 0.05% to 0.2% sorbates are required to combat gram negative bacteria such as Pseudomonas aeruginosa and Klebsiella species. 0.2% to 0.4% sorbates are required to fight Pseudomonas fluorescens.

Molds such as Candida albicans, Candida parapsilosis, Aspergillus species, Penicillum species, Fusarium species, Geotrichum candidum, Rhizopus nigricans, Pullularia pullulans, Rhodotorula rubra, and Alternaria species are kept in check by 0.05% to 0.2% sorbates.

Use Levels of Sorbic Acid and Potassium Sorbate in Cosmetics Market Survey, 1995

(According to M. Jager, 1995 Preservatech Conference Proceedings)

Product	Used w/ Chelating Agent	pH-Value	Concentration%[a]
Shampoo	Yes	4.8–5.6	0.15–0.3
Shower Gel	Yes	4.8–5.6	0.15–0.35
Body Lotion	Yes	5.0–6.0	0.1–0.2
Sun Lotion	Yes	5.2–5.6	0.1–0.2[b]
Cleansing Lotion	No	5.8–6.2	0.1–0.2[b]
Toning Lotion	Yes	5.8	<0.1[b]
Artificial Tanning Lotion	Yes	4.9	<0.1[b]
Oral Hygiene Products	No	6.5–6.6	0.15
Moist Tissues	Yes	5.5–5.9	0.1–0.15

[a] *Concentrations are calculated as sorbic acid, although potassium sorbate is more commonly used.*

[b] *Sorbic acid used in combination with other preservatives.*

Safety and Regulatory Status

Sorbic acid is a naturally occurring fatty acid similar in structure to corn oil's linoleic acid and margarine's oleic acid. Because sorbates are commonly used as preservatives for foods, they have been subjected to repeated toxicological testing. In acute oral toxicity studies, sorbic acid and potassium sorbate were practically nontoxic to mice and rats.

Sorbates do not irritate the skin. At concentrations up to 10%, sorbic acid and potassium sorbate were practically nonirritating to rabbits' eyes. Very few allergic reactions to sorbic acid have been demonstrated. As a result, sorbates are often used in baby-care products and creams and lotions.

Sorbic acid and potassium sorbate have been tested for mutagenic and other genotoxic effects using a variety of tests. The sorbates were at most weakly genotoxic in some of the tests.

Sorbates are nonphotosensitizing, so they are also appropriate as preservatives for sun care products.

Sorbates are environmentally safe. Even though they function as antimicrobials, they are rapidly and completely broken down in biological wastewater treatment plants. Sorbic acid is classified in the lowest water hazard class (0) by the German government and does not harm aquatic life. Many other cosmetic preservatives are classified in water hazard class 1 or 2. A few are even classified as a 3, the highest water-hazard class.

Sorbic acid and potassium sorbate have general acceptance as preservatives for almost all types of foods and are accepted for use in cosmetics in accordance with the International Cosmetic Ingredient Dictionary and Handbook, CTFA.[1]

- The CTFA Cosmetic Ingredient Review (CIR) panel has concluded that sorbic acid and potassium sorbate are safe as cosmetic ingredients in the present practices of use and concentration—up to 1.0%.
- The European Commission Cosmetic Directive has approved the use of sorbic acid without restrictions or warning labels at levels up to 0.6%. This is equal to 0.8% potassium sorbate.
- The Japanese Ministry of Health and Welfare has approved sorbic acid and potassium sorbate for use in hair-care products and cleansing, makeup, suntan and sunscreen, lip, eyeliner, and bath preparations at levels up to 0.5%. This level of sorbic acid is equal to 0.67% potassium sorbate.
- Sorbates have been approved as cosmetic preservatives in China and Australia.

[1]*Cosmetic, Toiletry, and Fragrance Association.*

Storage and Handling

Eastman sorbic acid and *Eastman* potassium sorbate are shipped and stored in boxes that have a moisture-barrier inner liner. The compounds deteriorate when exposed to heat or light for prolonged periods of time. Boxes should be kept closed as much as possible. Storage areas should be cool and dry. In order to minimize exposure to elevated temperatures, boxes should not be stored next to steam lines or directly under space heaters.

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e a s t m a n

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Spectrastat by Inolex

Product Bulletin

Spectrastat™ G2 Patent Pending

INCI ADOPTED NAME

Caprylhydroxamic Acid (and) Glyceryl Caprylate (and) Glycerin

GENERAL INFORMATION

Spectrastat G2 is a complete system for preservative-free cosmetic and personal care products. **Spectrastat G2** contains no biocides or typical preservatives. Instead it uses multifunctional ingredients that have excellent efficacy as *biostatic* and *fungistatic* agents.

Spectrastat G2 is ideal for personal care products where a paraben-free or preservative-free claim is needed. It can be used as an alternative to traditional preservative blends that are seen as undesirable by the consumer. A special benefit of **Spectrastat G2** is that it performs superbly at neutral pH, a state where many other alternative materials are ineffective.

Spectrastat G2 is compatible with most cosmetic ingredients. However, it can interact with residual iron found in some *clay-type compounds* (e.g., bentonite, silicates, etc). This interaction with iron may produce a very mild orange color or color shift that is barely perceivable to the eye in most formulations. In cases where the clays are high in iron, the colored compounds may be more perceivable.

PRINCIPAL USES

Spectrastat G2 may be used in emulsion, anhydrous, and surfactant systems. These include creams, lotions, shower gels, and make-up. It may be added to the water phase, at ambient or hot temperatures, or may be added post-emulsification of O/W emulsions.

During formulation/compounding, lengthy exposure to elevated temperatures should be avoided. For example, when compounding at 90°C, exposure should be limited to two hours; when compounding at 60°C, exposure should be limited to six hours.

Typical use level is 1.0% w/w to 1.2% w/w.

PHYSICAL PROPERTIES (TYPICAL)

Appearance .....Yellow to amber, Clear liquid above room temperature
Odor.....Mild, characteristic

STORAGE AND HANDLING

Store indoors, below 30°C and away from sources of heat. The product may solidify or precipitate. Gently heat to 35° – 45°C with mixing until material is homogeneous. It is recommended that normal safety precautions be employed when handling **Spectrastat G2**. Refer to the material Safety Data Sheet (SDS) for further information.

SAFETY DATA

Refer to the material Safety Data Sheet (SDS) for further information.

STANDARD PACKAGING

Plastic pail, 55 lb (24.9 kg) net weight.

2101 S. Swanson St. | Philadelphia, PA 19148 USA | toll free + 1 800 521 9891 | office + 215 271 0800 | fax + 215 271 6282 | www.inolex.com

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Weak-Acid Preservatives

Journal of Applied Microbiology 1999, **86**, 157–164

Weak-acid preservatives: modelling microbial inhibition and response

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R.J. LAMBERT AND M. STRATFORD. 1999. Weak-acid preservatives are widely used to prevent microbial spoilage of acidic foods and beverages. Characteristically, weak-acid preservatives do not kill micro-organisms but inhibit growth, causing very extended lag phases. Preservatives are more effective at low pH values where solutions contain increased concentrations of undissociated acids. Inhibition by weak-acids involves rapid diffusion of undissociated molecules through the plasma membrane; dissociation of these molecules within cells liberates protons, thus acidifying the cytoplasm and preventing growth. By modelling preservative action in yeast, using a thermodynamic and kinetic approach, it was possible to demonstrate that: (i) inhibition depends more on the degree to which individual preservatives are concentrated within cells, rather than on undissociated acid concentration *per se*; (ii) it is entirely feasible for microbes to pump protons out of the cell during extended lag phase and raise internal pH (pH_i), despite further influx of preservatives; (iii) the duration of the lag phase can be predicted from the model, using a Gaussian fit of proton-pumping H^+-ATPase activity against pH_i; (iv) theoretical ATP consumption for proton pumping can be directly correlated with the reduction in cell yield observed in glucose-limited cultures.

NOMENCLATURE

pH_i, internal (cytoplasmic) pH; pH_o, external (extracellular) pH; $[HA_o]$, external associated weak-acid concentration/mol l^{-1}; $[HA_i]$, internal associated weak-acid concentration/mol l^{-1}; $[A^-_i]$, internal dissociated, anion concentration/mol l^{-1}; $[A^-_o]$, external dissociated anion concentration/mol l^{-1}; K, weak acid equilibrium constant; r, rate of proton efflux, mol/time units; t, time elapsed, arbitrary time units.

INTRODUCTION

The documented use of weak-acid preservatives to inhibit growth of micro-organisms in foods and beverages extends back many centuries. John Evelyn in 1670 described the use of sulphur dioxide from burning sulphur in the preservation of cider (Rose and Pilkington 1989). Sulphur dioxide and sulphites continue to be the method of choice for the preservation of wine. Other weak-acid preservatives include acetic acid in pickles, propionic acid in bread and more recently, sorbic and benzoic acids in soft drinks (Chichester and Tanner 1972). All are targeted mainly against yeasts and moulds; low pH alone, less than pH 4·5, will prevent spore germination and growth of the great majority of bacteria (Gardner 1972; Smelt *et al.* 1982). Over the last few years, consumer demand for more 'natural' foodstuffs has caused a move away from chemical additions to food products and legislation in many parts of the world now limits their use. For example, within the EEC, sorbic acid is limited to 300 ppm in soft drinks. Preservative-resistant yeasts such as *Zygosaccharomyces bailii* can grow in soft drinks containing in excess of 500 ppm (Ingram 1960; Neves *et al.* 1994).

Weak-acid preservatives appear to share a common mode of action, despite their various chemical structures. All become increasingly potent as antimicrobial agents at more acidic pH values. In aqueous solution, weak-acids exist in pH-dependent equilibria between uncharged, acid molecules and their respective charged anions, for example acetic acid/acetate. The proportion of undissociated acid increases as the pH declines; the pH value at which there exists equal proportions of molecular acid and charged anions, is termed

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the pK_a. It is generally agreed that only undissociated acids have antimicrobial activity, although some activity by anions has been suggested (Eklund 1989).

Affected micro-organisms are rarely killed but growth is prevented. After very extended lag phases lasting days or even weeks, growth is poor and cell yields are greatly reduced. Inhibition of respiration and active transport have been reported (Freese *et al.* 1973). The mechanism of action of weak-acid preservatives is thought to involve diffusion of lipophilic acid molecules through the plasma membrane into the cytoplasm (Stratford and Rose 1986). There they encounter a pH value near to neutrality and are forced to dissociate into charged ions. Charged ions cannot return across the plasma membrane and anions are thus concentrated within the cell (Fig. 1). Dissociation of each weak-acid molecule will release a proton and the cytoplasm will become increasingly acidic. Acidification of the cytoplasm may prevent growth by inhibition of glycolysis (Krebs *et al.* 1983), by prevention of active transport (Freese *et al*. 1973) or by interference with signal transduction. pH_i is increasingly recognized as having a role in signalling (Thevelein 1994). The cellular response to inhibition caused by weak-acid preservatives may involve removal of preservatives by an efflux pump (Warth 1989), although evidence for this is disputed (Cole and Keenan 1987). Of greater importance is more likely the plasma membrane H^+-ATPase. This has been shown to be involved in weak-acid resistance (Cole and Keenan 1987; Vallejo and Serrano 1989), although its role remained questionable given that if pH_i were raised by proton pumping, further weak-acid molecules would penetrate the cell and re-acidify the cytoplasm.

Here, a model is presented based on known principles of physical chemistry, in which cytoplasmic pH is progressively raised during the lag phase by proton pumping, despite the influx of further weak acid. This model allows the prediction of the lag time required to raise the internal pH and for growth to begin.

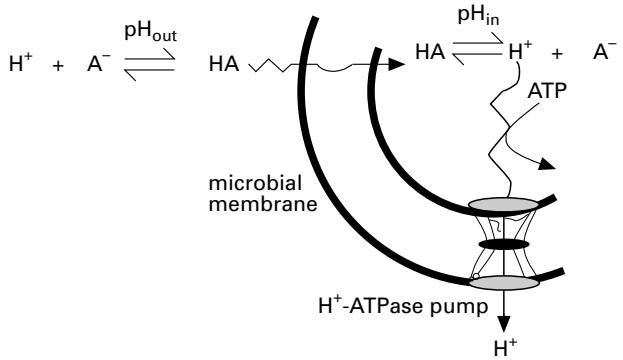

Fig. 1 Predicted medium and cytoplasmic weak-acid/anion equilibria. Only uncharged weak-acid molecules (HA) can diffuse freely across the plasma membrane. Charged anions (A^-) and protons (H^+) are retained within the cell; cytoplasmic protons are expelled by the membrane-bound H^+-ATPase

MATERIALS AND METHODS

Yeast strain

The yeast strain used in this work was *Saccharomyces cerevisiae* X2180–1B, **MAT**α *SUC2 mal gal2 CUP1*. This is available from the National Collection of Yeast Cultures, Institute of Food Research, Norwich NR4 7UA, UK, as strain NCYC 957.

Media and culture conditions

Yeast cultures were maintained at 4 °C on YEPD agar slopes. This contained glucose 20 g l^{-1}, yeast extract 10 g l^{-1}, bacteriological peptone 20 g l^{-1} and agar 20 g l^{-1}. Aerobically-grown, 24 h starter cultures were used to inoculate experimental cultures at 1 mg dry weight l^{-1} (approximately 10^4 cells ml^{-1}). As indicated, potassium sorbate was added to YEPD broth and the pH adjusted with HCl prior to autoclaving. In certain experiments, a semi-defined medium (pH 4·0) was used to minimize preservative binding. This contained fructose 20 g l^{-1}, ammonium sulphate 1 g l^{-1}, KH_2PO_4 3 g l^{-1}, citric acid 6 g l^{-1} and yeast extract 1 g l^{-1}. Preservatives were added from filter-sterilized 500 mmol l^{-1} stock solutions. The yeast was grown in 50 ml media aliquots in 125 ml conical flasks, at 30 °C, on an orbital shaker, 150 rev min^{-1}. Growth was monitored by optical absorbance at 600 nm and converted to dry weight using a calibration curve. The duration of the lag phase was estimated by linear regression of the semilog growth plots, and determining the intersection of the growth line with the log of the inoculum cell concentration.

Undissociated fractions of weak-acids

Proportions of dissociated and undissociated forms of weak-acid preservatives at each pH were calculated using the Henderson-Hasselbalch equation:

$$pH = pK_a + \log\frac{[A^-]}{[HA]}$$

Undissociated fractions of sulphite, nitrite, sorbate and benzoate are shown in Table 1.

Modelling pH_i and proton transport

The basic model. For the purpose of the model, activities are modelled as concentrations. This simplification holds true

Table 1 Percentage of undissociated acid/anions of weak-acid preservatives at pH values 4·0–6·75

pH	SO_2	Sulphite/ bisulphite	Nitrous acid	Nitrite	Sorbic acid	Sorbate	Benzoic acid	Benzoate
4·0	0·585	99·415	16·317	83·683	84·902	15·098	61·314	38·686
4·25	0·330	99·670	9·881	90·119	75·975	24·025	47·125	52·875
4·5	0·186	99·814	5·808	94·192	64·006	35·994	33·386	66·614
4·75	0·105	99·895	3·351	96·649	50·000	50·000	21·987	78·013
5·0	0·059	99·941	1·913	98·087	35·993	64·007	13·681	86·319
5·25	0·033	99·967	1·085	98·915	24·025	75·975	8·183	91·817
5·5	0·019	99·981	0·613	99·387	15·098	84·902	4·773	95·227
5·75	0·011	99·989	0·346	99·654	9·091	90·909	2·741	97·259
6·0	0·006	99·994	0·195	99·805	5·324	94·676	1·560	98·440
6·25	0·003	99·997	0·109	99·891	3·065	96·935	0·883	99·117
6·5	0·002	99·998	0·062	99·938	1·747	98·253	0·499	99·501
6·75	0·001	99·999	0·035	99·965	0·990	99·010	0·281	99·719

Values were calculated using the Henderson-Hasselbalch equation and pK_a values of SO_2/bisulphite, 1·77; nitrous acid/nitrite, 3·29; sorbic acid/sorbate, 4·74; benzoic acid/benzoate, 4·20.

for low concentrations. At higher concentrations, the individual concentrations should be replaced by activities.

Consider two vessels, 1 and 2, containing weak acid, at equilibrium, from the definition of the equilibrium constant, the following holds:

$$\frac{[H_1^+][A_1^-]}{[HA_1]} = \frac{[H_2^+][A_2^-]}{[HA_2]} \quad (1)$$

Consider now a situation where one of the vessels is the interior of a cell separated from the other by a semi-permeable membrane; Equation 1 must also be satisfied in an equilibrated system. Undissociated weak-acids are capable of diffusing freely through microbial membranes and do so until equilibrium is reached (Stein 1981; Stratford and Rose 1986). The equilibrium attained will satisfy Equation 1 and due to the free movement of the weak-acid across the membrane, $[HA_o] = [HA_i]$. The dissociated anion is not freely permeable and is therefore trapped inside the cell when the weak acid dissociates. This means that any difference in the pH between the internal and extracellular fluids will also be reflected in the concentrations of the dissociated anion. The assumption is made that the dissociated anion cannot leave the cell, and that the attainment of $[HA_o] = [HA_i]$ is faster than any other process linked to the model.

From the definition of the equilibrium constant:

$$-\log[H_o^+] - \log[A_o^-] + \log[HA_o] = -\log[H_i^+] - \log[A_i^-] + \log[HA_i] \quad (2)$$

From the definition of pH:

$$pH_o - \log[A_o^-] + \log[HA_o] = pH_i - \log[A_i^-] + \log[HA_i] \quad (3)$$

For the situation where $pH_o = pH_i$ and as, for a semi-permeable membrane, $[HA_o] = [HA_i]$, then $[A_o^-] = [A_i^-]$. If $pH_o \neq pH_i$ then Equation 4 must be satisfied:

$$\log\frac{[HA]}{[A_o^-]} - \log\frac{[HA]}{[A_i^-]} = pH_i - pH_o \quad (4)$$

With this model, a weak-acid has been added to a solution containing a microbe. The internal pH immediately falls and an equilibrium is reached such that the internal and external pH values are equal; this point is defined as time = 0. It is assumed that the diffusion of weak-acid into the cell is infinitely fast compared with any active proton pumping that may occur. The model consists of calculating the accumulation of anion coupled to the rate of proton efflux, and then using this value to calculate the internal pH (Equation 4).

Within the cell $HA \rightleftharpoons H^+ + A^-$.

Protons may be pumped from the cytoplasm by the H^+-ATPase. For every proton removed, one anion remains accumulated. HA then diffuses in through the membrane to immediately reset the equilibrium. However, as there are now 'extra' anions, the equilibrium concentrations required are slightly different and the internal pH alters. From Equation 4, at t = 0, Equation 5 is obtained, where $Q = \log[H_o^+][A_o^-]$.

$$\log \frac{[A_i^-]_{(t=0)}}{Q} = pH_{i,t=0} \quad (5)$$

The rate of proton efflux is equal to the rate of anion accumulation. Thus, the change in internal pH can be obtained from Equation 6, where r = rate of proton efflux, t = time elapsed.

$$pH_i = pH_{t=o} + \log\left(1 + \frac{rt}{[A_i^-]_{t=0}}\right) \quad (6)$$

Here, the rate of proton efflux is constant and independent of pH_i (anion accumulation is linear with time). On a longer time-scale, as the internal pH rises above 7, anion accumulation still occurs at the same rate. This is a system lacking feedback inhibition to the proton pump. As such this is not a realistic situation and the model requires adjustment. The modification involves limiting the rate of proton efflux with respect to the internal pH. A limiting factor, f, is introduced into Equation 6:

$$pH_i = pH_{t=0} + \log\left(1 + \frac{rft}{[A_i^-]_{t=0}}\right) \quad (7)$$

The limiting factor must regulate the output of the proton pump. For this regulation a pH is defined, the nominal pH, pH_n, at which the effectiveness of the proton pump is zero (i.e. stops pumping) and the effectiveness of the proton pump is also defined at pH_i, t = 0 (= pH_o) to be 100%. In this scenario, the protons are pumped out as fast as possible to begin with and then, as the internal pH rises, the pumping slows down until pH_n is reached. In this model, change in internal pH is calculated over short time intervals (Equation 8), and the changes in pH summed to give the internal pH (Equation 9).

$$\Delta pH_i = \log\left\{1 + \frac{r}{[A_i^-]_o}\left(\frac{pH_n - pH_i}{pH_n - pH_o}\right)\right\} \quad (8)$$

$$pH_i = pH_o + \Sigma\, \Delta pH_i \quad (9)$$

Modelling the H^+-ATPase function. To obtain a realistic model, the *in vivo* rate of H^+-ATPase activity with respect to pH should be used as the limiting factor. The efficiency of H^+-ATPase with respect to pH is known from experimental work (Willsky 1979; Eraso and Gancedo 1987). At low pH (<4·5), the enzyme was sluggish but achieved optimal performance at pH 5·5 (100% activity). At pH 7, it was shown to have 70% of optimum activity. Tests were carried out using isolated enzymes or membrane preparations. The work by Willsky (1979) gives activity at pH 10 which is obviously biologically unrealistic. In these tests, the enzyme lacked normal feedback inhibition mechanisms, and the operation of the H^+-ATPase would cease at some nominal pH because of feedback inhibition, except for enzyme used to maintain a pH to power active transport. The experimental data from low pH to optimum pH were fitted to half a Gaussian curve. The bold assumption was made that the feedback inhibition followed the other half of the Gaussian curve. This means that the efficiency of the H^+-ATPase approaches zero at low pH and also at the expected nominal pH (approximately pH = 7). The fit to the experimental data is portrayed in Fig. 2. The Gaussian expression for the efficiency of the enzyme is described in Equation 10:

$$\text{efficiency} = 10^{(-1/2(pH - pH_p/G_w))^2} \quad (10)$$

where pH_p = peak pH of the Gaussian curve; G_w = measure of the width of the curve. A Gaussian function with pH_p = 5·5 and G_w = 0·487 (parameters from experimental data) was used as the enzyme factor in Equation 7 and modelled using the analogous form of Equation 8.

RESULTS

Growth inhibition by preservatives

Yeast inhibition by sulphite, nitrite, sorbic and benzoic acids was compared. At pH 4·0, the undissociated fractions of these inhibitors were 0·58% SO_2, 16·3% nitrous acid, 84·9% sorbic acid and 61·3% benzoic acid (Table 1). In semi-defined medium containing increasing concentrations of preservatives, inhibition of yeast growth was found after 60 h in greater than 0·9 mmol l^{-1} SO_2/sulphite, 0·6 mmol l^{-1} nitrous acid/nitrite, 0·8 mmol l^{-1} sorbic acid/sorbate or 1 mmol l^{-1} benzoic acid/benzoate, at pH 4·0. In terms of undissociated acid, this is 5·3 μmol l^{-1} SO_2, 98 μmol l^{-1} nitrous acid, 613 μmol l^{-1}

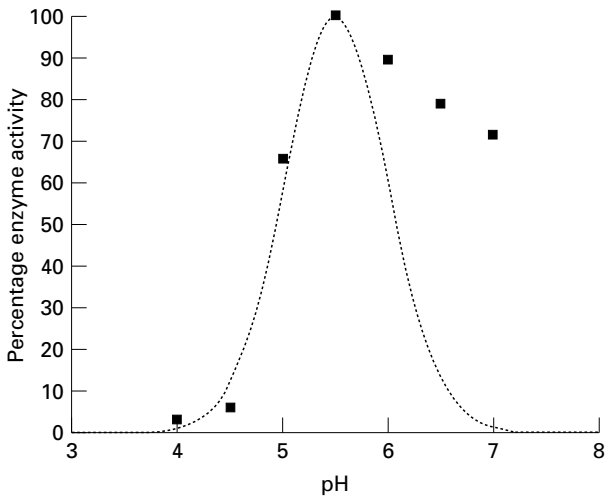

Fig. 2 Gaussian fit of the pH profile of the plasma-membrane H^+-ATPase (-----), based on the experimental data (■) of Willsky (1979)

benzoic acid or 679 μmol l^{-1} sorbic acid. Clearly, inhibition is not directly related to the concentration of undissociated acid in the medium.

However, undissociated acid is predicted to diffuse into the cell until the concentration is equal on both sides of the membrane. If the internal pH, pH_i, is maintained by buffering at pH 6·75 or restored to this level by proton pumping, the degree to which preservatives can be concentrated within the cell can be calculated for each pH value and preservative (Fig. 3). For example, sorbic acid/sorbate at pH 4·75 are in a 1:1 ratio (Table 1). Inside the cell at pH 6·75, the ratio is 1:100. As sorbic acid is at equal concentration on both sides of the membrane, the sorbate anion will be concentrated 100-fold within the cell. The overall preservative concentration outside is 1 + 1, and inside, 1 + 100, giving a concentration ratio of 1:50·5.

Figure 3 predicts that at pH 4, sorbate will be concentrated within the cell by $\times 86$, benzoate by $\times 218$, nitrite by $\times 466$ and sulphite by $\times 585$. If inhibition is a consequence of preservative uptake, then SO_2/sulphite should be most effective, followed by nitrous acid/nitrite, and sorbic acid/sorbate, benzoic acid/benzoate. Inhibitory concentrations of preservative show nitrous acid/nitrite to be marginally more effective than the others on a molar basis.

Modelling microbial response

If a microbial suspension is placed in a solution of weak-acid preservative, the internal pH will drop as weak-acids are freely permeable across microbial membranes. A possible response to this stress involves the removal of protons and consequent accumulation of anions. At first sight, raising pH_i through use of the H^+-ATPase appears to be a futile, ATP-wasting activity because a higher pH_i will cause a further influx of preservative and consequent lowering of pH_i. However, careful examination of the equilibrium shows that pH_i will not be lowered back to its original position. Proton pumping by the H^+-ATPase will raise the internal pH, albeit slowly and with great expense in terms of ATP. Figure 4 models the recovery of pH_i in the presence of three concentrations of the sorbic acid preservative, by proton pumping. Recovery is time-dependent on preservative concentration.

Calculating lag times

In the presence of a weak acid preservative, the time spent in the lag phase is increased (Table 2). Preliminary evidence suggests that to enter the exponential growth phase, the

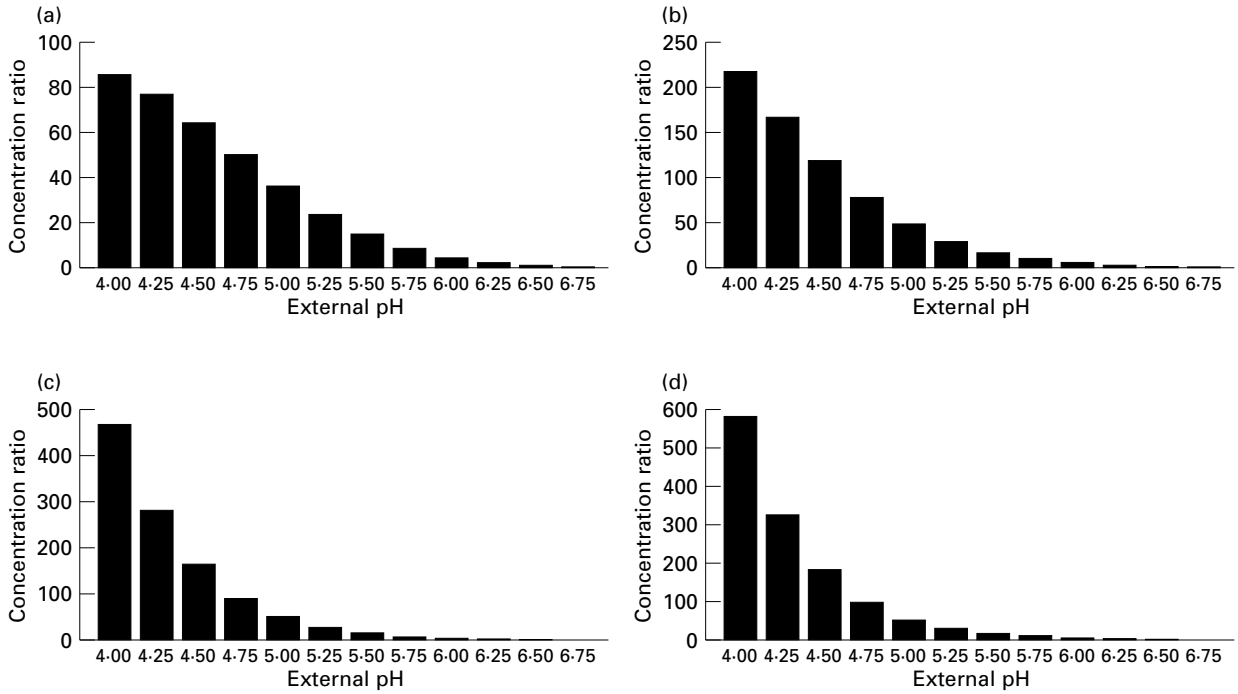

Fig. 3 Predicted concentration ratios of preservatives from medium to cytoplasm, based on known proportions of undissociated acid/anion at each pH value (Table 1). Concentrations are calculated assuming pH_i to be 6·75, due either to infinite buffering or to proton removal. (a) Sorbic acid/sorbate; (b) benzoic acid/benzoate; (c) nitrous acid/nitrite; (d) SO_2/sulphite

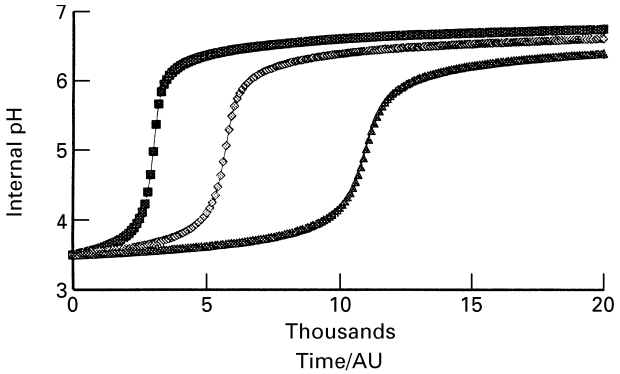

Fig. 4 Modelling the rise of pH_i from pH 3·5 by proton pumping, despite further weak-acid influx. Sorbic acid concentrations used were 0·5 mmol l^{-1} (■), 1 mmol l^{-1} (◇) and 2 mmol l^{-1} (▲). Time is in arbitrary units. Increased time is required (lag phase) to raise pH_i with increased preservative concentration

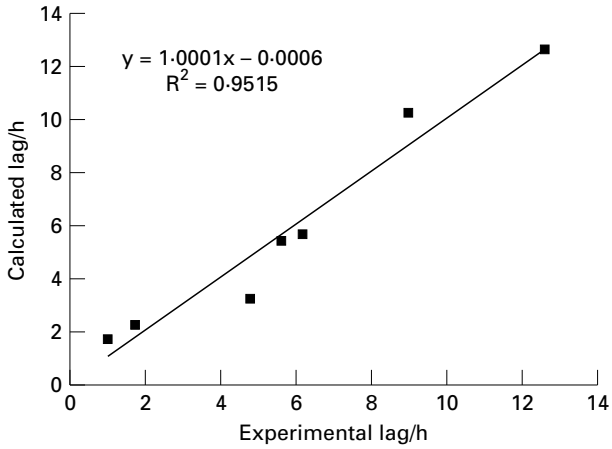

Fig. 5 Scatter plot of calculated and experimentally-determined lag phases of *Saccharomyces cerevisiae* X2180–1B

internal pH must be raised above a threshold value (Imai and Ohno 1995). Increasing the weak-acid concentrations may lead to increased lag times because the microbe has to pump out excess protons to achieve the required growth pH. The time taken to pump out this number is a direct reflection of the increased lag time observed. In the model shown here, the time taken to attain a specific internal pH (the threshold pH) would correspond to the end of lag time.

An internal pH of 5·8 was chosen as a reasonable estimate of the value for threshold pH. From the experimental results (Table 2), the extreme values for lag times were used to set the parameters of the Gaussian function. Using this fitted Gaussian, the time taken to reach an internal pH for a given pH and sorbic acid concentration was calculated (Table 2 and Fig. 5). In the model, the units of time are arbitrary. A correction (re-scaling) factor can be fitted to the time units as was done with the data in Table 2. Experimentally- and theoretically-derived lag times are in reasonable agreement. Figure 5 shows the calculated *vs* experimental data. The parameters used to fit the data are those for the H^+-ATPase of *Saccharomyces cerevisiae* given above ($pH_p = 5·5$, $G_w = 0·489$).

Calculating yields

If a microbe uses up energy reserves of ATP and sugars to combat the effect of a weak-acid preservative, when (or if) the microbe reaches the threshold internal pH, there will be less available for production of biomass. Physiologically, for every proton pumped out, one ATP is consumed. This model can equate the rate of protons pumped to the accumulation of anion. Therefore, the amount of anion accumulated over a set time interval reflects the ATP consumed, and therefore should relate to final biomass yield.

For this calculation, the Gaussian parameters used for the estimation of lag times are applied. However, instead of calculating the time taken to reach a specific internal pH, the amount of anion accumulated via proton efflux is calculated

Table 2 Duration of lag phase of *Saccharomyces cerevisiae* X2180-1B in YEPD containing sorbic acid at various pH values

Sorbic acid (mmol l^{-1})	pH 3·0	pH 3·3	pH 3·6	pH 3·9	pH 4·2	pH 4·5
3·0	—	—	—	—	—	16400 (20·5)
2·5	—	—	—	—	—	13700 (12·4)
2·0	—	—	—	15600 (16·7)	13600 (11·2)	11000 (6·9)
1·5	—	13700 (17·7)	12800 (12·0)	11700 (10·3)	10200 (5·4)	8300 (5·1)
1·0	9900 (9·9)	9100 (7·8)	8500 (5·6)	7800 (4·7)	6800 (2·7)	5500 (2·9)
0·5	5100 (4·3)	4600 (3·4)	4300 (3·4)	4000 (3·2)	3400 (2·3)	2700 (2·1)

Lag times were calculated from the model and are expressed in arbitrary time units. Experimental data are shown within brackets and expressed in hours. Control cultures lacking preservative grew with little or no lag (less than 0·2 h).

for a given time. For this study, yields (mg dry wt l^{-1}) are converted into a percentage yield loss. This normalizes the data with respect to the control yield. The experimental results and the modelled results are shown in Fig. 6, and demonstrate a good correlation.

DISCUSSION

Freese *et al.* (1973) examined the antimicrobial activity of a number of lipophilic weak-acids and noted a similarity of physiological effect on micro-organisms, despite their disparate chemical structures. Growth was inhibited as was active uptake of amino acids, organic acids and phosphate. All are likely to have a common cause, namely the lowering of the internal pH caused by weak-acids. Weak-acid preservatives have been shown to be concentrated within cells (Kotyk 1962; Macris 1975; Stratford and Rose 1986). As protons are released in a 1:1 molar ratio with anions within the cell, the degree of concentration is likely to reflect the relative toxicity of each preservative, all other factors being equal. Here, it is shown that while SO_2/sulphite and nitrous acid/nitrite were predicted to be most potent inhibitors (Fig. 3), in practice they showed a similar degree of inhibition to sorbic acid. Clearly, other factors impinge on weak-acid toxicity. Sulphite and nitrite may be lost due to oxidation (Hammond and Carr 1976). Sulphite is also known to be progressively detoxified by the production of binding compounds during the lag phase (Stratford *et al.* 1987). Alternatively, sorbic acid may be regarded as more toxic than expected. Secondary toxic actions for sorbic acid have been suggested, inhibiting glycolysis (Azukas *et al.* 1961) or acting on the plasma membrane (Stratford and Anslow 1996, 1998). However, an elongated lag phase did appear to be related to a weak-acid-type action by sorbic acid (Stratford and Anslow 1996).

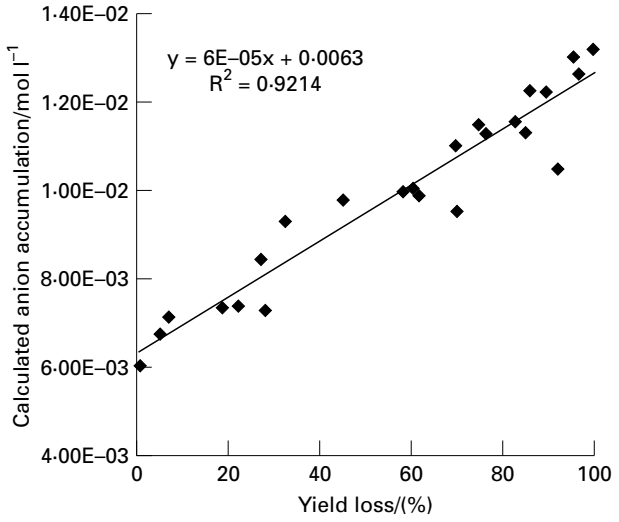

Fig. 6 Scatter plot of experimentally-determined loss of cell yield of *Saccharomyces cerevisiae* X2180–1B against calculated accumulation of anion. It is predicted that each anion accumulated represents expenditure of one ATP in proton extrusion. Hence, calculated ATP usage shows a linear relationship with yield loss

The model shown here of the changes in internal pH of cells afflicted by weak-acid preservatives are based only on known principles of physical chemistry and a Gaussian relationship of H^+-ATPase activity with pH. This demonstrates that it is entirely feasible to pump protons out of the cell, slowly raising pH_i, despite the consequent influx of more weak-acid. This can most easily be explained by the fact that for any given internal and external pH, there is a defined ratio of preservative concentrated in the cell (Fig. 3, Equation 4). If pH_i was raised and excess preservative entered the cell, pushing pH_i back to its previous position, more preservative would now be within the cell than permitted for this pH and it would no longer be in chemical equilibrium. Some preservative must then flow out, allowing pH_i to rise a little, thus restoring equilibrium. Proton pumping is therefore not a futile activity. This model also demonstrates that, having raised the pH_i to a level permitting growth, no further proton pumping is required. It is therefore unnecessary to postulate continuous pumping and ATP usage throughout growth, as had previously been suggested (Warth 1988).

In this model, for convenience, the assumption is made that there is no buffering capacity within the cell and the pH_i has also been allowed to fall to the external pH, following the addition of preservative. Optimum buffering is likely at pH 4·5–5·5 (Krulwich *et al.* 1985), and while the pH_i may not fall far, the proton pumping task will remain unaltered. Internal buffering will release the same number of protons, as the pH_i is raised again. Thus, this model is likely to reflect accurately the time taken to raise pH_i and thereby, the duration of the lag phase.

In addition to prolonging the lag phase, weak-acid preservatives are known to diminish cell yield in batch culture (Stratford and Anslow 1996). Experimentally, a relationship between the duration of the lag phase and the loss of cell yield can be shown. A good correlation was obtained (Fig. 6) between the experimental results and those calculated assuming that the usage of ATP in proton pumping is diverted from that used in growth. This gives credence to the model and also suggests that any other inhibitory action by sorbic acid does not involve the expenditure of ATP.

To conclude, using a thermodynamic and kinetic model, it is possible for weak-acid inhibited cells to raise pH_i by H^+-ATPase pumping. The time required to remove protons can be used to predict the duration of the lag phase and the calculated ATP expenditure is inversely proportional to experimentally determined biomass yields.

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