CHOOSING

Natural Preservatives

Manufacturer Information & Articles

Compiled by
Lise M Andersen

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Hello!

My name is Lise Andersen, owner of LisaLise Pure Natural Skin Care, author of LisaLiseBlog.com, and founder of Formulators Kitchen.

The most frequent question I get from my students, readers
and followers is
“Which is the best natural preservative?”

I wish there was a short and simple way to answer this, but there isn’t.
Choosing a preservative is always formula specific and can even change if as little as one ingredient is substituted.

This booklet is a compliation of
manufacturer information on some preservatives
that are generally accepted as ‘natural’. Some of them are COSMOS approved, some are ECOCERT approved, and some have no classification.

I have also included a few tips on what to consider
when choosing a preservative.

I hope this will be useful as a starting point and
help you in your research.

Lise Andersen
LisaLise Pure Natural Skin Care & Formulators Kitchen.

Contents

1.

Seven Things to Consider When Choosing a Natural Preservative

1. Define Natural

Not everyone has the same understanding of the term natural. One possiblity can be to choose ingredients that are either COSMOS or ECOCERT approved.

Some Ecocert / Cosmos Compliant ingredients used in preservation systems

- Benzoic acid (and its salts)
- Benzyl alcohol
- Dehydroacetic acid (and its salts)
- Salicylic acid (and its salts)
- Sorbic acid (and its salts)

Keep in mind that the listed ingredients will need to be combined with other ingredients in order to form a broad spectrum preservation system.

2. Consider pH

Each preservative has a pH range where it will function optimally. Be sure your chosen preservative is compatible with the pH of your product.

3. Consider Solubility

A preservative that is oil soluble may not be the best choice for an aqueous product. While it is not impossible to use, adding an oil solulbe preservative to an aqueous product such as a skin tonic will require a solubiliser in order to incorporate correctly and fully. It may be prefereable (and easier to work with) if you choose a water-soluble preservative.

4. Ingredient Compatibility

Some preservatives are incompatible with some ingredients (electrolytes, acids, etc). Be sure the preservative you select will function with the other ingredients in your formula. Issues that can arise include instability, change in viscosity, and change in color.

The manufacturer should have some initial information on this, but expect to do your own testing, regardless of how much information you have.

5. Packaging Compatibility

Some preservatives are not suitable for some packaging types. Example: aerosol packaging has specific requirements. Check that your chosen packaging is compatible with the preservative you select.

Be mindful as well that some preservatives are susceptible to photo-oxidation and are therefore not ideal for transparent packaging.

6. Usage & Overall Impact

Recommended usage rates vary widely in preservation systems - from 0.5% up to 4.0%. If your preferred preservative is expensive, adjusting the amount can impact your production costs - even if you are making artisan-sizaed batches.

Some natural preservatives have a rather strong inherent scent which will have to be taken into account as part of the overall fragrance profile of your product: for example a preservative containing benzyl alcohol can have a quite overpowering fragrance that some find difficult to work with.

7. Activity Range

The preservative needs to function so no pathogens or contaminants are allowed to spoil your product during its shelf life.

A broad spectrum preservation system will keep gram positive and gram negative bacteria as well as yeasts and moulds at bay.

A preservative that ‘only’ an antimicrobial is not broad spectrum. This is why broad spectrum preservatives are almost always a ‘cocktail’ of different ingredients to create a broad spectrum preservation system.

2.

Cosphaderm Sodium LAAS

Company: Cosphatec GmbH

INCI: Sodium Levulinate (and) Sodium Anisate

Description

White powder of Sodium Levulinate and Sodium Anisate.
This blend displays skin conditioning and masking properties, as well as a antimicrobial effectiveness.

TECHNICAL DATASHEET

COSPHADERM® SODIUM LAAS

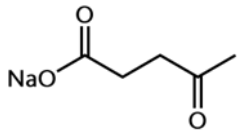
1.1 GENERAL INFORMATION

Trade name:	Cosphaderm® Sodium LAAS	
Item No.:	01-015-xxxx	
Supplier:	Cosphatec GmbH Hopfenmarkt 33 20457 Hamburg Germany	
Chemical name:	Sodium 4-Oxovalerate, Sodium 4-Methoxybenzoate	
INCI name:	Sodium Levulinate, Sodium Anisate	
CAS No.:	Sodium Levulinate	19856-23-6
	Sodium Anisate	536-45-8
EINECS/ EC No.:	Sodium Levulinate	243-378-4
	Sodium Anisate	208-634-1
IECIC:	Sodium Levulinate, -	

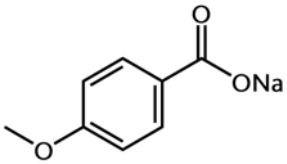
1.2 PRODUCT COMPOSITION

INCI name	CAS No.	%
Sodium Levulinate	19856-23-6	min 50
Sodium Anisate	536-45-8	25-50

1.3 CHEMICAL STRUCTURE



Sodium Levulinate



Sodium Anisate

1.4 PRODUCT SHORT DESCRIPTION

Cosphaderm® Sodium LAAS is a white powder comprising the solid mixture of Sodium Levulinate and Sodium Anisate. This blend displays skin conditioning and masking properties, as well as a broad antimicrobial effectiveness. This way, Cosphaderm® Sodium LAAS can help to ensure a formulations microbiological stability without the use of traditional preservatives.

The corresponding acids of both salts show a strong combined antimicrobial efficiency against bacteria, yeasts and moulds. The lower the pH value of the final formulation, the more the balance between salts and acids is shifted to the latter. Thus, it is essential to consider the pH of the formulation when using Cosphaderm® Sodium LAAS. Application within a pH range from 5.0 – 6.0 is possible, though 5.5 is ideal. Below a pH of 5.0, precipitation can occur, while above 6.0 the antimicrobial activity is diminished.

1.5 APPLICATION

Recommended concentration:	1.0 % (complete antimicrobial protection)
Conditions:	water soluble
Hints:	Add recommended concentration to the water phase of your formulation. Reduce the pH value at the end of your formulation to 5.0 – 6.0, ideally 5.5.

2.1 SPECIFIED DATA ANALYSED PER BATCH

Appearance:	White to slightly yellowish powder (visual)
Odour:	None to sweet flowery (organoleptic)
Solubility in water:	min. 100 g/L
Loss on drying:	max 5.0 %

2.2 SPECIFIED DATA ANALYSED PERIODICALLY

Heavy Metals:	max 10 ppm	
	Total Aerobic Count	max 100 cfu/g
	Total Yeasts and Moulds	max 50 cfu/g
Sum of Actives (dry basis)	min 98% (NMR)	

3.1 PHYSICAL AND CHEMICAL PROPERTIES

Molecular formula:	$C_5H_7NaO_3$ (Sodium Levulinate), $C_8H_7NaO_3$ (Sodium Anisate)
Molecular weight:	138.1 g/mol (Sodium Levulinate), 174.13 g/mol (Sodium Anisate)
Appearance:	white to slightly yellowish powder
Odour:	characteristic
Solubility:	water soluble

3.2 PACKING AND STORAGE CONDITIONS

Packing sizes:	20 kg cardboard box with 4 aluminium bags à 5 kg
Storage conditions:	Store in the air-tight original sealed containers protected from direct sunlight, moisture and heat at ambient temperature (15-25°C). Brief deviation from the storage temperature e.g. during transport does not impair product quality or shelf life.
Shelf life:	24 months, unopened and stored under proper conditions

4.1 REGULATORY COMPLIANCE

The product contains the specified ingredients Sodium Levulinate, Sodium Anisate.

Sodium Levulinate (CAS: 19856-23-6) is included/listed in the following international inventories:

EINECS (Europe), DSL (Canada); TCSI (Taiwan); NZioC (New Zealand); IECSC (China).

Sodium Anisate (CAS: 536-45-8) is included/listed in the following international inventories:

EINECS (Europe), NIoZ (New Zealand).

4.2 REGULATORY STATUS FOR COSMETIC APPLICATION

Product complies with Regulation (EU) No. 1223/2009 in all the defined requirements.

4.3 ADDITIVES

Product contains only the specified ingredients. No other substances like preservatives, antioxidants, fragrances, colourants or other are added.

4.4 IMPURITIES

	Not expected*	Present#	ppm
BSE/TSE	☒	☐	
Dioxin	☒	☐	
Formaldehyde	☒	☐	
Gluten	☒	☐	
Lactose	☒	☐	
Pesticides	☒	☐	
Phthalates	☒	☐	
Residual solvents	☒	☐	

Heavy metals		ppm
	total	≤ 10
	Lead (Pb)	≤ 1
	Arsenic (As)	≤ 1

4.5 ALLERGENS

Product does not contain any of the 26 allergenic flavours or fragrances listed in Regulation (EC) No. 1223/2009 Annex III.

4.6 VOC

Product does not contain any volatile organic compounds.

4.7 CMR

Product is not rated as CMR (category 1A, 1B and 2) and does not contain any ingredient rated as CMR according to Regulation (EC) No. 1223/2009 in association with Regulation (EC) No. 1272/2008/EC.

4.8 SVHC

Product is neither listed as SVHC substances nor contains any material which is listed as SVHC substance.

4.9 NANOMATERIAL

Product is not embraced by article 16 Regulation (EC) No. 1223/2009.

4.10 ANIMAL TESTING

The Cosphatec GmbH has not conducted any animal testing for cosmetic purpose since March 11th 2009.

4.11 IRRADIATION

Product has not been irradiated in any step of the entire production process.

4.12 ORIGIN

Substances (INCI name)	Synthetic	Vegetal	Biotechnology
Sodium Anisate	☐	☒	☐
		Name of the plant: Star anise *(Illicium verum)* Plant part used: Branches, leaves and fruits	Microorganism used:

		Origin: China	
Sodium Levulinate	☐	☒ Levulinic Acid neutralized by the presence of NaOH Levulinic Acid: Name of the plant: Sugar cane Origin: China	☐ Microorganism used

4.13 GMO

The product is not subject to labelling according to Regulations (EC) No. 1829/2003 and 1830/2003.

4.14 CERTIFICATIONS

COSMOS

OTHER INFORMATION

This information and our technical application advice regarding use and handling are given to the best of our knowledge, but they are for information purposes only. Cosphatec GmbH bears no responsibility for deviated use or handling of the products. Customers or manufacturers are not released from their responsibility to perform own testing particularly with regard to the qualification of our products for intended processes and purposes. The sale of our products occurs according to our current general sales terms and delivery conditions.

* not expected to be present due to raw materials, production process and used equipment. Not regularly analysed.

expected to be present due to raw materials, production process and used equipment. Periodically analysed.

3.

Verstatil BOB

Company: Evonik

INCI: Benzyl alcohol, Caprylyl Glycol, Benzoic Acid

Description

Safe and mild preservation system offering broad antimicrobial function.

Verstatil® BOB

An optimized blend of multifunctional additives and preservatives with excellent broad antimicrobial activity. Verstatil® BOB is suitable for all types of cosmetic products within a wide pH range. It allows the formulator to work without criticized preservatives.

INCI

Benzyl Alcohol; Caprylyl Glycol; Benzoic Acid

Benefits at a glance

- Balanced mixture of two well-known preservatives (benzyl alcohol and benzoic acid) combined with the boosting activity of the wetting agent Caprylyl Glycol
- No phenoxyethanol/ isothiazolinone/ parabens/ formaldehyde-releasers/ halogenorganic compounds
- Safe and mild preservation, standalone solution
- Low impact on the stability and appearance of the formulation
- For all types of cosmetic products
- Globally approved

Product form

Liquid

Applications

AP/Deo
After Sun
Body Care
Body Cleansing
Color Cosmetics
Eye Care
Face & Neck Care
Facial Cleansing
Hair Care
Hair Cleansing
Hair Conditioning & Hair Treatments
Hand & Foot Care
Lip Care
Mens Care
Scalp Care
Skin Care
Skin Cleansing
Sun Care
Sun Protection

Safety Data Sheets

4.

Feniol

Company: Sinerga

INCI: Phenethyl Alcohol and Caprylyl Glycol

Description

Liquid preservative with distinctive rose fragrance

Sinerga
SKIN EVOLUTION

SCIENTIFIC RESEARCH | RAW MATERIALS | THIRD PARTY MANUFACTURER

Feniol

" Free" claims-friendly preservative

FOCUS INFO

INCI NAME

Phenethyl Alcohol (and) Caprylyl Glycol

SPECIFICATIONS

Appearance	clear liquid
Colour:	colourless
Odour:	characteristic (mild rose-like odour)
Phenetyl alcohol:	55 - 65%
Capryl Glycol:	35 – 45%
Density (at 25°C):	0.97 – 0.98 g/ml
Dosage:	0.5 – 1.5 %

COSMETIC APPLICATIONS

- Effective preservative system to create mild and safe self-preserving formulations
- Suitable for creams, oils and rinse-off products such as shampoo, shower gel and bath
- Sensitive skin and baby care products

CHARACTERISTICS

Feniol is a well balanced compound effective against Gram positive, Gram negative bacteria, yeasts and moulds.
It has a wide spectrum antimicrobial activity and represents an **alternative to traditional cosmetic preservatives**, allowing to create **self-preserving formulations** with reduced irritating and sensitizing potential. It gives a **pleasant mild-rose aroma** to the final product.
Feniol does not contain preservatives listed in EU Annex VI or allergens and allows the claims **preservative-free** and **fragrance-free.**

PROPERTIES

- Broad spectrum antimicrobial activity
- Approved worldwide in all cosmetic applications
- Allows to create self-preserving formulations
- Reduced irritating and sensitizing potential
- Easy to handle and formulate

Sinerga S.p.A. Via della Pacciarna, 67 - 21050 Gorla Maggiore (VA) Italy Ph. +39 0331 16031 Fax +39 0331 1603401
www.sinerga.it

Sinerga
SKIN EVOLUTION

SCIENTIFIC RESEARCH · RAW MATERIALS · THIRD PARTY MANUFACTURER

Feniol

ANTIMICROBIAL ACTIVITY

Inhibitory (MIC) and Biocidal (MBC) activity of FENIOL in ppm

Test organism (10^6 CFU/mL)	Minimum Inhibitory concentration (MIC ppm)	Minimum Biocidal concentration (MBC ppm)
Gram-positive bacteria *S. aureus ATCC 6538*	**2500**	**3500**
Gram-negative bacteria *E. coli ATCC 8739* *P. aeruginosa ATCC 9027*	**1750** **3000**	**3500** **4000**
Yeasts *C. albicans ATCC 10231*	**2500**	**5000**
Moulds *A. niger ATCC 16404*	**1750**	**7000**

CHALLENGE TEST TRIALS

Formulation	FENIOL %	Results after 7 days
NON IONIC O/W EMULSION	1.0	ADEQUATE
O/W EMULSION GEL	0.8	ADEQUATE
O/W EMULSION GEL	1.0 + EDTA 0.1%	ADEQUATE
SHOWER BATH	1.0 + EDTA 0.1%	ADEQUATE

HEAVY LEGS CREAM

INGREDIENTS	PHASE	w/w %
HITECREAM® (Potassium Palmitoyl Hydrolyzed Oat Protein, Behenyl Alcohol, Palm Glycerides, Sodium Stearoyl Glutamate, Sucrose Palmitate)	A	7,50
Cetearyl Isononanoate	A	5,00
Dicaprylyl Carbonate	A	4,00
Coco-Caprylate	A	3,50
Tocopherol	A	q.s.
Water	B	0,15
Glycerin	B	0,50
Mycrocrystalline cellulose, Cellulose Gum	B	1,00
Escin, Beta-sitosterol, Phospholipids	B	3,00
50°C Menthol	C	0,10
Squalane	C	0,50
XSOLVE (Ethyl Ximenynate, Lecithin)	E	2,00
Parfum	F	2,00

METHOD
A - Dose A heat to 70°C. B – As the same time dose B and heat to 70°C. Add A to B C – Cool down and at 30°C add C-F stirring very slowly

*Formulation tested in Sinerga Research Centre according to stability and laboratory manufacturing procedures.

CODICE- REV 09/2017

Sinerga S.p.A. Via della Pacciarna, 67 - 21050 Gorla Maggiore (VA) Italy Ph. +39 0331 16031 Fax +39 0331 1603401
www.sinerga.it

FENIOL

Solution for self-preserving formulations

Chemical nature Combination of Phenylethyl alcohol in Octane-1,2-diol.

INCI name	CAS N°	EINECS N°	Composition
Phenethyl alcohol	60-12-8	200-456-2	55-65%
Caprylyl glycol	1117-86-8	214-254-7	35-45%

Physico-chemical data		
	Appearance:	Clear liquid
	Color:	Colourless
	Density (at 25°C):	0.970-0.980 g/ml

Microbiological specifications		
	Bacteria:	≤ 100 cfu/g
	Moulds and yeasts:	≤ 10 cfu/g
	P. aeruginosa:	absent
	S. aureus:	absent
	C. albicans:	absent

Sinerga S.p.A.
Direzione, Uffici, Centro Ricerca e Unità Produttiva
Via della Pacciarna, 67 – 21050 Gorla Maggiore (VA) - Italia
Tel +39 0331 16031 Fax +39 0331 1603400-401
E-mail: info@sinerga.it
www.sinerga.it

Sede Legale:
Via A. Bertani, 6
20154 Milano - Italia
C.C.I.A.A. 1600680
Partita Iva IT12950420153
Cap.Soc € 500.000,00 i.v.

Stability Product is stable if stored in normal conditions.

Compatibility Generally compatible with all substances. Any incompatibility could be due to single ingredients of the mixture. It could be easily incorporated in oil-based formulations too.

Indications Well balanced combination of widely accepted and safe cosmetic ingredients which has a synergistic antimicrobial action with a broad spectrum protection of cosmetics against all classes of micro-organisms.

Intended uses In emulsions should be incorporated with stirring after the emulsification at temperature below 50°C.

Suggested dosage It can be used at concentrations between 0.5-1.5%. A potentiating effect is obtained in combination with Disodium EDTA.

Storage Keep product in a cool place away from direct light. Store in an air-tight container. The product may crystallize after prolonged exposure to temperature near 0°C, anyway it can be easily re-solubilized when brought back to room temperature.

Preservative system Product is unpreserved.

Toxicological data

1. Skin Irritation (*in vitro*): minimum irritant.
2. Ocular Irritation (*in vitro)*: mild irritant.

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Via della Pacciarna, 67 – 21050 Gorla Maggiore (VA) - Italia
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E-mail: info@sinerga.it
www.sinerga.it

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20154 Milano - Italia
C.C.I.A.A. 1600680
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Cap.Soc € 500.000,00 i.v.

5.

Geogard ECT

Company: Arxada

INCI: Benzyl Alcohol & Salicylic Acid & Glycerin & Sorbic Acid

Description

Broad spectrum preservation

arxada

GEOGARD® ECT

Geogard® ECT

Broad Spectrum Preservation System

Preservation

INCI Name: Benzyl Alcohol & Salicylic Acid & Glycerin & Sorbic Acid

Recommeded Use Level: 0.6–1.0%

Description

Geogard® ECT is a unique, patented combination of benzyl alcohol, salicylic acid, glycerin and sorbic acid, which are well accepted in a wide range of personal care products. The novel composition of this antimicrobial blend offers a low cost in use as well as broad spectrum protection in a diverse range of products against Gram-positive and Gram-negative bacteria, yeast and molds at wide pH ranges. Geogard® ECT has a wide range of global regulatory acceptance for personal care products.

Key Product Benefits

- Broad spectrum activity on bacteria, yeast and molds
- Low odor profile; Ideal for fragrance-free and fragrance-sensitive systems
- Wide pH compatibility: pH 3-8
- Excellent safety profile
- COSMOS & Soil Association approved
- Vegan
- Non-GMO
- Cruelty-free [Not Tested On Animals]
- China compliant

Compositional Breakdown

Chemical Compound	CAS No.	EINECS No.	%
Benzyl Alcohol	100-51-6	202-859-9	77-86%
Salicylic Acid	69-72-7	200-712-3	8-15%
Glycerin	56-81-5	200-289-5	3-6%
Sorbic Acid	110-44-1	203-768-7	1-4%

Applications


Body Care


Hair Care


Makeup


Skin Care

Typical Properties

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

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

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.

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%

Hair Conditioner 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	3.5×10^5	<10	<10	<10	<10	<10	<10	<10
K. pneumoniae + *E. gergoviae*	9.4×10^5	3.4×10^5	2.6×10^8	3.5×10^6	<10	<10	<10	<10
P. aeruginosa + *B. cepacia*	4.9×10^5	$>10^6$	3.0×10^8	<10	2.0×10^2	<10	<10	<10
C. albicans	3.3×10^5	3.3×10^6	2.7×10^6	2.8×10^7	6.0×10	<10	<10	<10
Mixed molds	2.1×10^4	3.5×10^3	1.2×10^3	1.4×10^4	<10	<10	<10	<10

Make-Up Remover (pH 5.15)

% water: 90%; A_w: 0.980

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

Make-Up Remover 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	9.0×10	<10	<10	<10	2.0×10	<10	<10	<10
K. pneumoniae + *E. gergoviae*	5.3×10^3	<10	<10	<10	4.0×10	<10	<10	<10
P. aeruginosa + B. cepacia	3.3×10^5	1.8×10^6	1.4×10^6	7.7×10^6	1.0×10	<10	<10	<10
C. albicans	1.8×10^4	1.9×10^4	1.2×10^4	1.5×10^4	<10	<10	<10	<10
Mixed molds	1.5×10^4	2.4×10^4	1.1×10^4	7.0×10^4	<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%

Make-Up Remover 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	1.0×10^2	<10	<10	<10	<10	<10	<10	<10
K. pneumoniae + *E. gergoviae*	5.1×10^6	8.0×10^6	2.5×10^6	8.0×10^5	<10	<10	<10	<10
P. aeruginosa + B. cepacia	4.5×10^6	6.6×10^6	1.5×10^6	3.2×10^6	<10	<10	<10	<10
C. albicans	4.0×10^2	<10	<10	<10	<10	<10	<10	<10
Mixed molds	1.1×10^4	2.5×10^4	2.0×10^4	1.0×10^5	<10	<10	<10	<10

Water in Oil Emulsion Cream (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%

Water in Oil Emulsion Cream 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	8.6×10^4	<10	<10	<10	<10	<10	<10	<10
K. pneumoniae + *E. gergoviae*	5.6×10^4	<10	<10	<10	<10	<10	<10	<10
P. aeruginosa + B. cepacia	3.1×10^4	2.9×10^3	<10	3.4×10^5	<10	<10	<10	<10
C. albicans	4.6×10^4	1.3×10^4	2.9×10^3	5.3×10^4	<10	<10	<10	<10
Mixed molds	1.2×10^4	9.7×10^3	7.0×10^3	3.4×10^5	<10	<10	<10	<10

Lotion (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%

Lotion 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	1.3×10^6	1.6×10^4	3.0×10^4	8.0×10^3	7.0×10	<10	<10	<10
K. pneumoniae + *E. gergoviae*	1.3×10^6	9.5×10^5	7.0×10^5	2.3×10^3	2.0×10	<10	<10	<10
P. aeruginosa + B. cepacia	$>10^6$	8.5×10^6	4.3×10^7	9.8×10^7	<10	<10	<10	<10
C. albicans	1.1×10^5	1.0×10^5	9.0×10^5	1.5×10^5	8.7×10^3	<10	<10	<10
Mixed molds	2.3×10^6	9.0×10^4	1.6×10^4	7.0×10^4	1.8×10^3	<10	<10	<10

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
  - There are restrictions in using salicylic acid in products for children under the age of 3

Japan

- Benzyl alcohol is not permitted for use as a preservative in final cosmetic products placed on the Japanese market, however it can be used as a cosmetic ingredient

United States

- All ingredients allowed (CIR/PCPC)
- Refer to present practices of use and concentration

China

- China compliant; listed on both the IECSC & IECIC inventories

General

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

GEOGARD® ECT

arxada

Arxada
412 Mt. Kemble Avenue, Suite 200S
Morristown, NJ 07960
Tel +1 800-365-8324
arxada.com

arxada.com

6.

Geogard Ultra

Company: Arxada

INCI: Gluconolactone & Sodium Benzoate

Description

Broad spectrum preservation

arxada

GEOGARD Ultra®

Geogard Ultra® (Patented)
Next-Generation Preservation

Preservation

INCI Name: Gluconolactone (and) Sodium Benzoate

Recommended Use Level: 0.75–2.0%

Description

A synergistic blend of gluconolactone and sodium benzoate, providing broad spectrum protection and ease of formulation. Typically organic acids on their own provide only anti-fungal protection and are too weak, requiring a co-preservative or booster to perform optimally. The glucanolactone in this blend works together with sodium benzoate to act as an efficient booster that also delivers moisturization to the end application providing true multifunctional benefits. This preservative system is the ideal choice for the naturally-minded formulator.

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

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%

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.

Applications


Baby Care


Hair Care


Deoderants


Wipes


Sun Care


Body Care


Skin Care


Color Cosmetics

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	Geogard Ultra® @1.5%
Total:	100.00%

Bacterial Counts (CFU/gram)

Sample#	Test Samples	Day 0	Day 7	Day 14	Day 28
1	Unpreserved Moisturizer	9.5×10^{6}	4.2×10^{5}	8.9×10^{4}	<10
2	Moisturizer with 1.5% Geogard Ultra®	6.5×106	<10	<10	<10

Fungal Counts (CFU/gram)

Sample#	Test Samples	Day 0	Day 7	Day 14	Day 28
3	Unpreserved Moisturizer	8.8×10^{5}	1.7×10^{5}	1.9×10^{5}	2.8×10^{5}
4	Moisturizer with 1.5% Geogard Ultra®	2.1×10^{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	Geogard Ultra® @1.5%
Total	100.00%

Bacterial Counts (CFU/gram)

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

Fungal Counts (CFU/gram)

Sample#	Test Samples	Day 0	Day 7	Day 14	Day 28
3	Unpreserved Shampoo	6.6×10^{5}	2.0×10^{5}	3.0×10^{5}	1.7×10^{7}
4	Shampoo with 1.5% Geogard Ultra®	4.4×10^{5}	<10	<10	<10

Hair Conditioner

(pH = 4.89)

Ingredient	% W/W
Water, deionized	q.s
Glycosperse 0-20 – Polysorbate 80	0.5%
Lecithin - Alcolec F100	1.0%
Distearyldimonium Chloride (Varisoft TA100)	2.0%
Cetyl Alcohol - CO-1695	2.1%
Cetearyl Alcohol -TA-1618	1.5%
Ethosperse LA-4 - POE 4 Lauryl Alcohol	3.1%
10% Aqueous Sodium Hydroxide	pH adjuster
Preservative	Geogard Ultra® @1.5%
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 Liqour

(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

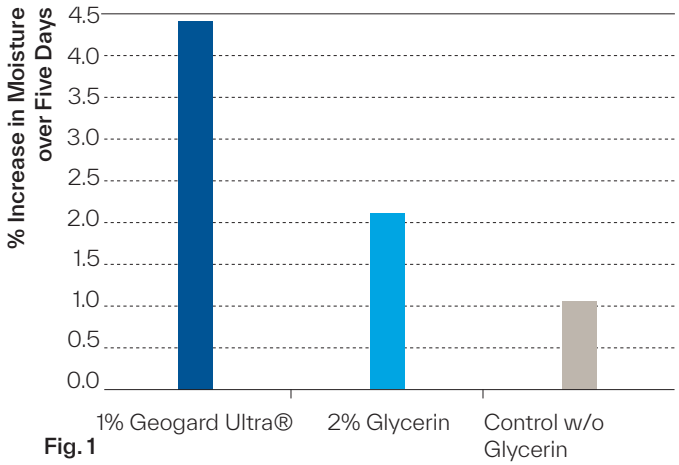

Fig. 1

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

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%

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

GEOGARD Ultra®

Arxada
412 Mt. Kemble Avenue,
Suite 200S Morristown, NJ 07960
Tel +1 800-365-8324
arxada.com

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7.

Iscaguard 9088

Company: Isca

INCI: Benzyl alcohol, dehydroacetoc acid, aqua

Description

Broad spectrum preservation in a blend of organic acids

Iscaguard 9088

Paraben Free	Thiazolinone Free	Formaldehyde Free	Preservative Free	Natural
✓	✓	✓		

INCI declaration
benzyl alcohol, dehydroacetic acid, aqua

Iscaguard 9088 is a blend of an organic acid in benzyl alcohol. It is suitable for formulations with acidic pH, and offers protection against bacteria, yeasts, and moulds. Iscaguard 9088 is permitted for use with most eco label certified formulations and can be used for both leave-on and rinse-off products.. The use of benzyl alcohol based preservatives offers excellent headspace protection for cosmetics supplied in susceptible packaging.

In Use Concentrations	ISCA recommendation	EU Cosmetic Regulation (max)
Leave-on	0.5 – 1.0 %	1.14 %
Rinse-off	0.5 – 1.0 %	1.14 %

Not to be used in aerosol dispensers (sprays) .

In use concentrations vary according to the formulation type and the other ingredients present. The correct use dosage should be determined by microbial challenge testing of the finished formulation (ISCA UK offers discounted challenge testing to our customers).

Recommended Applications

Shampoo, Shower gel (Rinse-off)	Creams, lotions (Leave-on)	O/W emulsions	W/O emulsions	Wet wipes	Eye care	Lip care	Oral care	Children under 3
●	●	●	●	●	●	●	●	●

Use scenarios derived from evaluation of Cosmetic Regulation guidelines and preservative performance for typical formulations.

Registered office: Nine Mile Point Industrial Estate, Cross Keys, Newport NP11 7HZ
Registered No: 3500179

Iscaguard 9088

Formulation guidelines	
pH (effective range)	3.0 – 6.0
Solubility (Water)	~1.0 %
Solubility (Glycols)	Soluble
Maximum Process Temperature	60 °C
General information	Iscaguard 9088 is compatible with most personal care ingredients. In aqueous formulations, heating to 40°C may be required in order to fully dissolve the preservative. However, prolonged exposure to high temperatures should be avoided in order to protect against discolouration. The preservative efficacy of Iscaguard 9088 increases as the pH value drops, so for optimal efficacy we recommend that the pH of the finished product is as low as possible. Iscaguard 9088 is not to be used in aerosol dispensers (sprays) according to the EU Cosmetic Products Regulation.

Minimum Inhibitory Concentrations	
Microorganism	MIC (%)
Bacteria (gram-negative)	
Pseudomonas aeruginosa	0.40
Escherichia coli	0.40
Bacteria (gram-positive)	
Staphylococcus aureus	0.40

Minimum Inhibitory Concentrations	
Microorganism	MIC (%)
Yeasts	
Candida albicans	0.15
Moulds	
Aspergillus brasiliensis	0.15

Registered office: Nine Mile Point Industrial Estate, Cross Keys, Newport NP11 7HZ
Registered No: 3500179

8.

Iscaguard 9256

Company: Isca

INCI: Benzyl alcohol, caprylyl glycol

Description

Broad spectrum preservation : a blend of organic acids in benzyl alcohol

Iscaguard 9256

Paraben Free	Thiazolinone Free	Formaldehyde Free	Preservative Free	Natural
✔	✔	✔	✗	✗

INCI declaration
Benzyl Alcohol, Caprylyl Glycol

Iscaguard 9256 is a liquid preservative blend containing Caprylyl Glycol and Benzyl Alcohol. It is a mild, yet effective preservative for personal care formulations that is highly effective against bacteria, yeasts, moulds and fungi. Iscaguard 9256 has the added benefit of headspace protection due to the presence of Benzyl Alcohol.

In Use Concentrations	ISCA recommendation	EU Cosmetic Regulation (max)
Leave-on	0.5 – 1.0 %	1.1 %
Rinse-off	0.5 – 1.0 %	1.1 %

In use concentrations vary according to the formulation type and other ingredients present. The correct use dosage should be determined by microbial challenge testing of the finished formulation (ISCA UK offers discounted challenge testing to our customers).

Recommended Applications

Shampoo, Shower gel (Rinse-off)	Creams, lotions (Leave-on)	O/W emulsions	W/O emulsions	Wet wipes	Eye care	Lip Care	Oral care	Children under 3
●	●	●	●	●	●	●	●	●

Use scenarios derived from evaluation of Cosmetic Regulation guidelines and preservative performance for typical formulations.

Formulation guidelines	
pH (effective range)	3.0 – 8.5
Solubility (Water)	~ 3 %
Solubility (glycols)	Miscible
Maximum Process Temperature	80 °C
General information	Iscaguard 9256 is compatible with most personal care ingredients. In aqueous formulations, heating to 40°C may be required in order to fully dissolve the preservative.

Minimum Inhibitory Concentrations	
Microorganism	MIC (%)
Bacteria (gram-negative)	
Pseudomonas aeruginosa	0.30
Escherichia coli	0.40
Bacteria (gram-positive)	
Staphylococcus aureus	0.30

Minimum Inhibitory Concentrations	
Microorganism	MIC (%)
Yeasts	
Candida albicans	0.40
Moulds	
Aspergillus niger	0.25

Physical Properties (approximate)	
Appearance	Colourless liquid
Odour	Mild aromatic
Density at 20°C	~ 1.035 gcm^{-3}

Physical Properties (approximate)	
Colour	< 20 Hazen
Solubility in water	~ 3 %
Solubility in glycols	Miscible

Safety information

Cosmetic Regulation labelling requirements
No special labelling requirements.

Transport information	
	not regulated
UN number	-
UN proper shipping name	-
Transport hazard class	-
Packing group	-
Environmental hazards	-

Hazard classification/labelling	
Hazard pictograms	
Signal word	Warning
Hazard statements	H319 Causes serious eye irritation. H302 Harmful if swallowed. H332 Harmful if inhaled.

ISCA Preserving the tradition

Chemical Business Association

ISCA UK Ltd Nine Mile Point Industrial Estate, Cross Keys, Newport NP11 7HZ, Wales, UK

Tel: +44(0)1495 200747 Fax: +44(0)1495 200757 E-mail: enquiries@iscauk.com **www.iscauk.com**

9.

Iscaguard BOA

Company: Isca

INCI: Benzyl alcohol, dehydroacetoc acid, benzoic acid, sorbic acid

Description

Broad spectrum preservation: blend of organic acids in benzyl alcohol

Iscaguard BOA

Paraben Free	Thiazolinone Free	Formaldehyde Free	Preservative Free	Natural
✔	✔	✔	✗	✗

INCI declaration
Benzyl alcohol, dehydroacetic acid, benzoic acid, sorbic acid

Iscaguard BOA is a blend of organic acids in benzyl alcohol. It is suitable for formulations with acidic pH, and offers protection against bacteria, yeasts, moulds and fungi. Iscaguard BOA is permitted for use with most Eco labels, and can be used for both leave-on and rinse-off products.

In Use Concentrations	ISCA recommendation	EU Cosmetic Regulation (max)
Leave-on	0.5 – 1.0 %	1.25 % #
Rinse-off	0.5 – 1.0 %	1.25 % #

Not to be used in aerosol dispensers (sprays)

In use concentrations vary according to the formulation type and other ingredients present. The correct use dosage should be determined by microbial challenge testing of the finished formulation (ISCA UK offers discounted challenge testing to our customers).

Recommended Applications

Shampoo, Shower gel (Rinse-off)	Creams, lotions (Leave-on)	O/W emulsions	W/O emulsions	Wet wipes	Eye care	Lip Care	Oral care	Children under 3
●	●	●	●	●	●	●	●	●

Use scenarios derived from evaluation of Cosmetic Regulation guidelines and preservative performance for typical formulations.

Formulation guidelines	
pH (effective range)	3.0 – 6.5
Solubility (Water)	1.0 % (approx.)
Solubility (Glycols)	Soluble
Maximum Process Temperature	40 °C
General information	Iscaguard BOA is compatible with most personal care ingredients. In aqueous formulations, heating to 40°C may be required in order to fully dissolve the preservative. However, prolonged exposure to high temperatures should be avoided in order to protect against discolouration. Iscaguard BOA works effectively at pH values below 6.5, though for optimal efficacy we recommend that the pH of the finished product is less than 6. Iscaguard BOA is not to be used in aerosol dispensers (sprays) under the Cosmetic Regulation.

Minimum Inhibitory Concentrations	
Microorganism	MIC (%)
Bacteria (gram-negative)	
Pseudomonas aeruginosa	0.25
Escherichia coli	0.06
Proteus vulgaris	0.24
Bacteria (gram-positive)	
Staphylococcus aureus	0.21
Bacillus cerus	0.21
Enterococcus faecium	0.21

Minimum Inhibitory Concentrations	
Microorganism	MIC (%)
Yeasts	
Candida albicans	0.13
Saccharomyces cerevisiae	0.11
Moulds	
Aspergillus niger	0.13

Physical Properties (approximate)	
Appearance	Light yellow liquid
Odour	Faint
Density	1.07 gcm^{-3}

Physical Properties (approximate)	
Flash point	96°C
Solubility in water	1.0 %
Solubility in glycols	Soluble

Safety information

Cosmetic Regulation labelling requirements
No special labelling requirements.

Transport information	
	not regulated
UN number	-
UN proper shipping name	-
Transport hazard class	-
Packing group	-
Environmental hazards	-

Hazard classification/labelling	
Hazard pictograms	
Signal word	Danger
Hazard statements	H318 Causes serious eye damage. H302 Harmful if swallowed H332 Harmful if inhaled H315 Causes skin irritation. H373 May cause damage to lungs through prolonged or repeated exposure.

ISCA Preserving the tradition

Chemical Business Association

ISCA UK Ltd Nine Mile Point Industrial Estate, Cross Keys, Newport NP11 7HZ, Wales, UK

Tel: +44(0)1495 200747 Fax: +44(0)1495 200757 E-mail: enquiries@iscauk.com **www.iscauk.com**

10.

Aquaguard NK12

Company: Isca

INCI: Aqua, sodium benzoate, potassium sorbate

Description

Broad spectrum preservation

Aquaguard NK12

Paraben Free	Thiazolinone Free	Formaldehyde Free	Preservative Free	Natural
✔	✔	✔	✗	✗

INCI declaration
Aqua, Sodium Benzoate, Potassium Sorbate

Aquaguard NK12 is an aqueous preservative suitable for use with both rinse-off and leave-on products. It has a broad spectrum of activity and is effective against bacteria, yeast, mould and fungi. The preservative is supplied as an easy to use, readily dispersible liquid which is alcohol and solvent free. Aquaguard NK12 is designed for use in skin friendly formulations with pH values up to 5.5.

In Use Concentrations	ISCA recommendation	EU Cosmetic Regulation (max)
Leave-on	1.0 – 1.9 %	1.96 %
Rinse-off	1.0 – 3.0 %	5.35 %

In use concentrations vary according to the formulation type and other ingredients present. The correct use dosage should be determined by microbial challenge testing of the finished formulation (ISCA UK offers discounted challenge testing to our customers).

Recommended Applications

Shampoo, Shower gel (Rinse-off)	Creams, lotions (Leave-on)	O/W emulsions	W/O emulsions	Wet wipes	Eye care	Lip Care	Oral care	Children under 3
●	●	●	●	●	●	●	●	●

Use scenarios derived from evaluation of Cosmetic Regulation guidelines and preservative performance for typical formulations.

Formulation guidelines	
pH (effective range)	2.0 – 5.5
Solubility (Water)	Fully soluble
Solubility (Glycols)	Fully soluble
Maximum Process Temperature	80 °C (avoid prolonged periods at high temperature)
General information	Aquaguard NK12 is compatible with most personal care ingredients. It is suitable for use in low pH formulations, but will lose efficacy if the pH rises above 5.5. The product as supplied may change in appearance from yellow to brown on storage, but no further discolouration occurs when it is used in the end product as long as the formulation remains acidic.

Minimum Inhibitory Concentrations	
Microorganism	MIC (%)
Bacteria (gram-negative)	
Pseudomonas aeruginosa	0.60
Escherichia coli	0.20
Bacteria (gram-positive)	
Staphylococcus aureus	0.30
Enterococcus hirae	0.30
Bacillus subtilis	0.05
MRSA	0.02

Minimum Inhibitory Concentrations	
Microorganism	MIC (%)
Yeasts	
Candida albicans	0.50
Saccharomyce cerevisiae	0.05
Saccharomyces rouxii	0.15
Rhodotolura sp	0.05
Moulds	
Penicillium funiculosum	0.05

Physical Properties (approximate)	
Appearance	Clear yellow to brown liquid
Odour	Mild odour
Density	1.176 gcm^{-3}
pH value	7.5 - 10.0

Physical Properties (approximate)	
Solubility in water	Fully soluble
Solubility in glycols	Fully soluble
VOC content	0 %

Safety information

Cosmetic Regulation labelling requirements
No special labelling requirements.

Transport information	
	IMDG
UN number	-
UN proper shipping name	Not dangerous goods
Transport hazard class	-
Packing group	-
Environmental hazards	-

Hazard classification/labelling	
Hazard pictograms	
Signal word	Warning
Hazard statements	H315 Causes skin irritation. H319 Causes serious eye irritation.

ISCA Preserving the tradition

Chemical Business Association

ISCA UK Ltd Nine Mile Point Industrial Estate, Cross Keys, Newport NP11 7HZ, Wales, UK
Tel: +44(0)1495 200747 Fax: +44(0)1495 200757 E-mail: enquiries@iscauk.com **www.iscauk.com**

11.

Lexgard Natural MB

Company: Inolex

INCI: Glyceryl Caprylate and Glyceryl Undecylenate

Description

Vegan friendly, COSMOS approved, primaruily efective against bacteria and yeast, with some efficacy against mould.

It is recommended used at concentrations of 1.0-1.5% w/w and is stable and effective at pH 4.0 - 8.0. Should be added to water phase during cool down or during emulsification process.

Technical & Regulatory Product Dossier

Lexgard® Natural MB

Last modified: July 12, 2019

GENERAL PRODUCT INFORMATION

INCI NAME	Glyceryl Caprylate (and) Glyceryl Undecylenate
CAS NUMBER	26402-26-6 (Glyceryl Caprylate) 65684-27-7 (Glyceryl Undecylenate)

PHYSIOCHEMICAL INFORMATION.

COMPOSITION	Glyceryl Caprylate – 90% Glyceryl Undecylenate – 10%
COUNTRY OF ORIGIN	United States
MANUFACTURING PROCESS	Glyceryl caprylate and glyceryl undecylenate are blended. The product is then filtered and packaged.
STATEMENT OF ORIGIN	Lexgard® Natural MB is derived from vegetable sources only. Glycerin and caprylic acid are derived from coconut and/or palm oil sourced from South East Asia. Undecylenic acid is formed directly from the pyrolysis of non-GMO castor oil sourced from India.

REGULATORY & COMPLIANCE INFORMATION

1223/2009 COMPLIANCE	• Complies with Annexes III, IV, V and VI, of the Cosmetic Regulation (EC) 1223/2009 • Does not contain any substance listed in Annex II of the Cosmetic Regulation 1223/2009, in the limit of Article 17 • Complies with Article 15 ("Substances classified as CMR substances") of the Cosmetic Regulation 1223/2009
ALLERGEN STATEMENT *FRAGRANCE*	Does not contain any of the allergens listed in Annex II or III of Cosmetic Regulation (EC) 1223/2009.
FOOD	Does not contain tree nuts, peanuts, soybeans, wheat, eggs, milk, fish, or crustacean shellfish.
ANIMAL TESTING STATEMENT	Has not conducted, nor commissioned, animal testing in accordance with Regulation (EC) No. 1223/2009, Chapter V; Article 18 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products.
BSE/TSE STATEMENT	Does not contain any animal derived ingredients, thus is Bovine Spongiform Encephalopathy (BSE)/Transmissible Spongiform Encephalopathy (TSE) free with respect to source, manufacture, and treatment.

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Technical & Regulatory Product Dossier

Lexgard® Natural MB

Last modified: July 12, 2019

CALIFORNIA PROPOSITION 65 STATEMENT

Not expected to contain any contaminants or by-products known to the State of California to cause cancer or reproductive toxicity as listed under Proposition 65 State Drinking Water and Toxic Enforcement Act.

CITES STATEMENT

No components are included on the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) list.

CMR STATEMENT

Not expected to contain C (Carcinogen), M (Mutagen), or R (Toxic for Reproduction) Substances as indicated on REACH Annex VI or in Regulation (EC) No. 1272/2008 (categories 1A, 1B, or 2).

GENETIC MODIFICATION STATEMENT

Not expected to contain genetically modified material; Starting raw materials do not intentionally include genetically modified organisms (GMOs) and no GMO materials are introduced during the manufacturing process.

GLUTEN-FREE STATEMENT

Not expected to contain gluten; Starting raw materials do not contain gluten and no gluten is introduced during the manufacturing process.

HALAL STATEMENT

- Does not contain any ingredient of animal origin
- Does not contain ethyl alcohol and ethyl alcohol has not been used in the manufacturing process
- The equipment used for manufacturing the product is not used for the manufacturing of products containing ingredients of animal origin
- Does not come in contact with any products of animal origin or products containing such ingredients

HEAVY METALS STATEMENT

Ag	As	Bi	Cd	Cr	Cu
<1 ppm	<1 ppm	<1 ppm	<1 ppm	<1 ppm	<1 ppm
Fe	**Hg**	**Mo**	**Pb**	**Sb**	**Sn**
<1 ppm	<1 ppm	<1 ppm	<1 ppm	<1 ppm	<1 ppm

IMPURITIES STATEMENT

The following list of chemicals are not expected to be present in the INOLEX family of products based on our knowledge of the starting raw materials used and the current manufacturing processes – This includes strict controls of raw materials, optimized synthesis processes for the production of new chemical entities, and the use of analytical testing to ensure the quality of the finished chemicals (though not all of the below chemicals are routinely analyzed).

- Oxides (butylene oxide, ethylene oxide, propylene oxide, etc.)
- Amines (melamine, nitrosamines, etc.)
- Glycol ethers
- Silicone
- Dioxane
- Formaldehyde
- Polyaromatic Hydrocarbons
- Halogenated compounds
- Phthalates
- Parabens
- Sulfates

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Lexgard® Natural MB

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Please note that the above list of chemicals is not an exhaustive list.

IRRADIATION STATEMENT

No known irradiated ingredients present and has not been subject to irradiation of any kind during or after manufacturing.

MICROBIOLOGICAL TESTING STATEMENT

Inherently resistant to microbial contamination.

NANOMATERIAL STATEMENT

Nanomaterials in accordance with Article 16 of Cosmetic Regulation (EC) 1223/2009 are not used in the manufacturing or processing and thus are not expected to be present.

PALM STATUS

INOLEX Inc. is a member of the Roundtable for Sustainable Palm Oil (RSPO), procures goods from suppliers that are also members of the RSPO, and can currently offer Mass Balanced versions of palm-derived products.

PESTICIDE STATEMENT

Not expected to contain residual pesticides; Starting raw materials are sourced from pesticide-free plants and no pesticides are introduced during the manufacturing process.

PRESERVATIVES/ADDITIVES STATEMENT

No preservatives or additives are present.

REACH COMPLIANCE STATUS

INCI Name	ECHA Substance Name	CAS No.	EINCS No.	Registration No.	REACH Deadline
Glyceryl Caprylate	octanoic acid, monoester with glycerol	26402-26-6	247-668-1	01-2120119773-55-0000	2018
Glyceryl Undecylenate	10-undecanoic acid, monoester with 1,2,3-propanetriol	65684-27-7	918-906-8	Exempt (<1t/year)	2018

RESIDUAL MONOMER STATEMENT

Not applicable.

RESIDUAL SOLVENT STATEMENT

Lexgard® Natural MB conforms with both the USP <467> and ICH Q3C residual solvent guidelines. INOLEX Inc. assures that, based raw on the raw material composition, the manufacturing, handling, and storage procedures utilized at our plant sites, there is no potential for residual solvents of class 1, 2, or 3 as mentioned in the guidelines for residual solvents (CPMP/ICH/283/95) of the European Pharmacopoeia, to be present in Lexgard® Natural MB.

SVHC STATEMENT

Does not contain Substances of Very High Concern (SVHC) under REACH.

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Technical & Regulatory Product Dossier

Lexgard® Natural MB

Last modified: July 12, 2019

VEGAN STATEMENT	Suitable for vegans; Derived from vegetable sources only and does not contain any animal derived ingredients.
VOC STATEMENT	Does not meet the definition as a Volatile Organic Compound (VOC) under any of the following regulations: • 40 CFR Part 51 Section 51.100 • California Air Resources Board's definition of reactive organic gas (ROG) or total organic gases (TOG) • EC Directive 1999/13/EC (Solvent Emissions Directive) • The Swiss Federal Council (based on 35a and 35c of the Environmental Protection Act)

TOXICITY REVIEW

ACUTE ORAL TOXICITY (Public Domain – REACH Registration Dossier)	**Glyceryl Caprylate (CAS #: 26402-26-2)** LD50 was found to be greater than 5000 mg/kg bodyweight. **Glyceryl Undecylenate (CAS #: 65684-27-7)** LD50 > 5,000 mg/kg bw
ACUTE DERMAL TOXICITY (Public Domain – REACH Registration Dossier)	**Glyceryl Caprylate (CAS #: 26402-26-2)** LD50 > 2 000 mg/kg bw
SKIN SENSITIZATION (INOLEX Study – RIPT	**Lexgard® Natural MB (INCI: Glyceryl Caprylate (and) Glyceryl Undecylenate)** The upper back between the scapulae served as the treatment area on 52 human subjects. Approximately 0.02 grams of Lexgard® Natural MB was applied to the 3/4" x 3/4" gauze portion of a clear adhesive dressing that was dampened with water. It was then applied to the treatment site to form a semi-occluded patch. This procedure was followed three times a week for a total of nine applications. Two weeks following the ninth application, a challenge patch was applied to the original site and to a virgin site. Each site was evaluated at 24 and 48 hours after application. Observations on all subjects remained negative throughout the test interval. Under the conditions of this study, **Lexgard® Natural MB did not indicate a potential for eliciting dermal irritation and/or sensitization**.
SKIN IRRITATION (INOLEX Study – EpiDerm MTT Viability Assay)	**Lexgard® Natural MB (INCI: Glyceryl Caprylate (and) Glyceryl Undecylenate)** Dermal toxicity or irritation potential of the test substance is determined by the ET50 for MTT viability of EpiDerm samples. MatTek EpiDerm tissue samples were treated with test substance followed by viability testing of the tissues using MTT uptake and conversion. Resulting absorbance of each sample was measured at 540 nm and the viability was then expressed as a percentage versus the control values. The calculated ET50 represents the time at which the EpiDerm tissue viability was reduced by 50% compared to the control tissues. ET50 for **Lexgard® Natural MB** > **2.2 hours and is therefore classified as a moderate irritant.**
EYE IRRITATION	**Lexgard® Natural MB (INCI: Glyceryl Caprylate (and) Glyceryl Undecylenate)**

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Lexgard® Natural MB

Last modified: July 12, 2019

(INOLEX Study – EpiOcular MTT Viability Assay)

Ocular toxicity or irritation potential of the test substance is determined by the ET50 for MTT viability of EpiOcular samples. MatTek EpiOcular tissue samples were treated with test substance followed by viability testing of the tissues using MTT uptake and conversion. Resulting absorbance of each sample was measured at 540 nm and the viability was then expressed as a percentage versus the control values. The calculated ET50 represents the time at which the EpiOcular tissue viability was reduced by 50% compared to the control tissues. **ET50 for Lexgard® Natural MB > 2.1 min and is therefore classified as a severe irritant**.

BIODEGRADATION (INOLEX Study – OECD 301A)

Lexgard® Natural MB (INCI: Glyceryl Caprylate (and) Glyceryl Undecylenate) Readily biodegradable

REGULATORY STATUS

	Lexgard® Natural MB	
	Glyceryl Caprylate (CAS #: 26402-26-6)	**Glyceryl Undecylenate (CAS #: 65684-27-7)**
Europe	REACH: Registered	Reach exempt
Canada	Listed on NDSL	Complies
Australia	Listed on AICS	Listed on AICS
Philippines	Listed on PICCS	Listed on PICCS
China	Listed on IECSC and IECIC	Listed on IECSC and IECIC

CERTIFICATIONS

COSMOS approved.

STORAGE

STORAGE CONDITIONS

It is recommended that INOLEX Inc. products be stored in unopened, original containers and be kept indoors.

RECOMMENDED RE-EVALUATION DATE

Recommended re-evaluation date 36 months. The recommended re-evaluation date is the time-period in which the product is expected to maintain its initial physical and chemical characteristics from the Date of Manufacture as indicated on the Certificate of Analysis. The recommended re-evaluation date period will be affected by storage conditions such as temperature, humidity, and the environment of the storage area.

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12.

Naticide

Company: Sinerga

INCI: Parfum

Description

Clear liquid with inherent vanilla-like scent.

Naticide® is nature-identical with broad spectrum activity.
Effective against Gram+, Gram-, yeasts and molds in a pH range between 4 - 9.

Sinerga
SKIN EVOLUTION

Scientific research · Raw materials · Third party manufacturer

Naticide®

Natural preservation

Preservative-free claims

FOCUS INFO

INCI NAME

Parfum

SPECIFICATIONS

Appearance:	clear liquid
Colour:	from colorless to pale yellow
Odour:	characteristic
Suggested dosage:	0.3% - 1 %
China Approved	

COSMETIC APPLICATIONS

Naticide® can be used in wide range of personal care formulations such as:

- Body and face creams
- Delicate detergents
- Cleansers

CHARACTERISTICS

Naticide® is a vegetable origin fragrance, created by Sinerga Research Centre, with a wide and complete spectrum activity, effective against Gram+, Gram-, yeasts and moulds in a pH range between 4 - 9.
Thanks to its vanilla-like aroma, the use of additional perfumes can be avoided and Naticide® could be also the base for a fresh and natural scent.
It is safe and extremely skin compliant.
A new concept for safe and preservative-free formulations of cosmetics according to the most innovative market trends.

PROPERTIES

- Full spectrum activity
- Preservative-free claims
- Excellent stability
- Total compatibility
- Fragrance fixative properties
- Great skin tolerability
- Light sweet smell
- Easy to handle

Sinerga S.p.A. Via della Pacciarna, 67 - 21050 Gorla Maggiore (VA) Italy Ph. +39 0331 16031 Fax +39 0331 1603401
www.sinerga.it

Sinerga SKIN EVOLUTION

SCIENTIFIC RESEARCH | RAW MATERIALS | THIRD PARTY MANUFACTURER

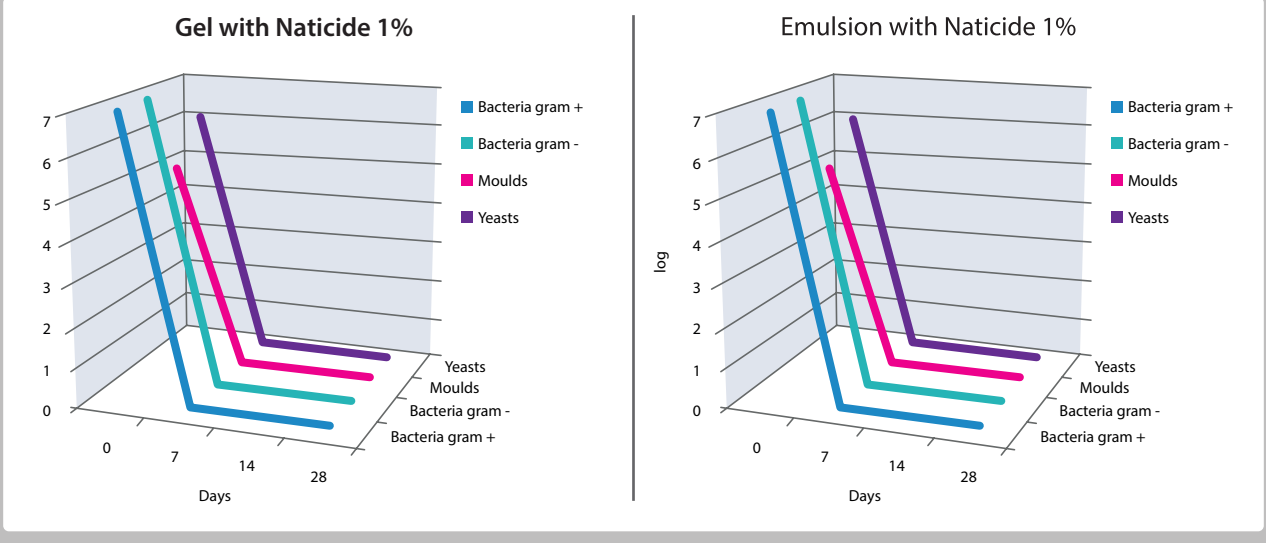
Naticide®

ANTIMICROBIAL ACTIVITY

The antimicrobial activity of Naticide® has been tested by a simulated microbial attack, better known as CHALLENGE TEST. The method use for the Viable count is the one according to Farmacopea Ufficiale Italiana IX ed. and the UE Policy 76/768 (dated 27.07.1976).

CHALLENGE TEST TRIALS

To determinate the microbial survival a total viable count has been performated after 7, 14, 28 days.



CHALLENGE TEST RESULTS

After 7 days from the inoculation, a drastic decrease of the microbial populations (-99,9%) has been registered with no further growth after 14 and 28 days.
Naticide® can be thus considered a high performance growth inhibitor, with high efficacy against all tested micro organisms.

FORMULATING WITH NATICIDE®

When formulating emulsions, add 1% Naticide® at the end.
Naticide® is water disperdible only up to 0.6%. In case of higher percentage, the remaining amount is to be added at the end of the formulation.
It is reccomended the usage of proper solubilizing agent into the hydrophilic phase.

6000000027- REV 09/2017

Sinerga S.p.A. Via della Pacciarna, 67 - 21050 Gorla Maggiore (VA) Italy Ph. +39 0331 16031 Fax +39 0331 1603401
www.sinerga.it

MICROBIAL INHIBITOR

NATICIDE®

Product category	Microbial Inhibitor.	
Chemical nature	Vegetable derived ingredient, obtained with a confidential procedure, with a wide spectrum of activity, being effective against Gram+, Gram-, yeasts and molds in a pH range between 4 - 9. Thanks to its vanilla-like scent, the use of additional perfumes can be avoided. Formulated according to IFRA recommendations.	
Applications	Body and face creams, delicate detergents and cleansers. Personal care formulations. Naticide is suitable for preservative-free formulations.	
INCI name	Parfum.	
Chemical-physical characteristics	Appearance: Colour: Odour:	Clear liquid. From colorless to pale yellow. Characteristic.
Microbiological specifications	Bacteria: Molds and yeasts: *P. aeruginosa*: *S. aureus*: *C. albicans*:	≤ 100 cfu/g ≤ 10 cfu/g absent absent absent
Formulative indications	Naticide® works better in a pH range between 4 and 9. Naticide® Dispersible in water up to 0.6%; complete in glycole and alcohol. In order to obtain a complete dispersion, add Naticide® under vigorous stirring. When formulating emulsions, add Naticide® amounts in both the hydrophilic and lipophilic phase.	

Sinerga S.p.A.
Direzione, Uffici, Centro Ricerca e Unità Produttiva
via della Pacciarna, 67
21050 Gorla Maggiore (VA) - Italia
Tel +39 0331 16031
Fax +39 0331 1603400-(401)
www.sinerga.it
info@sinerga.it

Sede Legale:
Via A Bertani, 6
20154 Milano - Italia
C.C.I.A.A. 1600680
Partita Iva IT12950420153
Cap.Soc € 500.000,00 i.v.

SOGGETTA ALL'ATTIVITA' DI DIREZIONE E COORDINAMENTO DELLA SINERGA GROUP srl

MICROBIAL INHIBITOR

Suggested dosage	From 0.3% to 1%.	
Stability	Product is stable when stored in normal conditions.	
Compatibility	Compatible with all the substances normally used in cosmetics.	
Storage	Keep product away from direct light. Store in an air-tight container.	
Toxicological data	Eye irritation (Het cam test sol.1%):	Hypoirritant
	Eye irritation (*in vitro*):	Moderate irritant
	Skin irritation (*in vitro*):	Minimum irritant
	Skin irritation (*in vivo* Patch-test sol.2%):	Non-primary irritant
	Skin sensitization (*in vivo* Patch-test sol. 2%):	Non-primary sensitizer
	UV Phototoxicity (*in vitro*):	Non phototoxic/ Non photoirritating
Shelf life	18 months	
HS code	3302 90 90	

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21050 Gorla Maggiore (VA) - Italia
Tel +39 0331 16031
Fax +39 0331 1603400-(401)
www.sinerga.it
info@sinerga.it

Sede Legale:
Via A Bertani, 6
20154 Milano - Italia
C.C.I.A.A. 1600680
Partita Iva IT12950420153
Cap.Soc € 500.000,00 i.v.

SOGGETTA ALL'ATTIVITA' DI DIREZIONE E COORDINAMENTO DELLA SINERGA GROUP srl

13.

Nipaguard SCE

Company: Clariant

INCI: Sorbitan caprylate, propanediol, benzoic acid

Description

Yellowish liquid, broad spectrum, ECOCERT, approved for oral care

Product Fact Sheet
NIPAGUARD® SCE

CLARIANT

Preservative for the cosmetic industry

Chemical name	Preservative blend consisting of Sorbitan Monooctanoate, 1,3-Propanediol and Benzoic Acid
INCI designation	Sorbitan Caprylate (and) Propanediol (and) Benzoic Acid

CLARIANT INTERNATIONAL LTD

Rothausstrasse 61
4132 Muttenz
Switzerland

BUSINESS UNIT INDUSTRIAL & CONSUMER SPECIALTIES

www.ics.clariant.com
www.clariant.com

PRODUCT PROPERTIES[1]

Appearance (20℃)	Yellowish to yellow colored liquid

Chemical and physical data

Gardner Colour	max. 8
Density (20 ℃)	1.12 g/cm^3
Sorbitan Caprylate	approx. 65 % w/w
Propanediol	approx. 20 % w/w
Benzoic Acid	approx. 15 % w/w

Uses

Nipaguard® SCE is a broad spectrum antimicrobial agent comprising a synergistic blend of Benzoic Acid in Velsan® SC (INCI: Sorbitan Caprylate) and Propanediol; designed for preservation of a wide range of cosmetics and toiletries. This blend is part of Clariant EcoTain range and is Ecocert certifiied, which makes it ideal for preservation of natural cosmetic products.

Regulatory status

Europe: Nipaguard® SCE can be used up to a maximum concentration of 3.1 % in leave-on products and practically no limitations for rinse-off (15.6%) and Oral Care (10.6%). No further restrictions according to Annex V of regulation (EC) No 1223/2009 have to be considered.

Japan: Maximum concentration 1.33 % for all kinds of cosmetic products.

USA: No restrictions, the ingredients of Nipaguard® SCE are designated “safe as used”.

Applications

Typical use concentrations of Nipaguard® SCE are 0.5 – 1.5 % for most cosmetic formulations. Nipaguard® SCE provides activity against gram positive and gram

[1] These characteristics are for guidance only and not to be taken as product specifications. The tolerances are given in the product specification sheet. For further product properties, specifications, safety and ecological data, please refer to the MSDS.

negative bacteria, yeasts and moulds. It works best at low pH from 4-5, but has also significant performance up to a pH of 6.5.

Incorporation

As a liquid Nipaguard® SCE is easily incorporated into formulations to be preserved. The solubility in water is below 0.05 g/l (20°C) and it is readily miscible with many organic solvents, surfactants and emulsifiers.

pH stability

Nipaguard® SCE remains fully stable from pH 4.0 – 8.0.

Temperature stability

Nipaguard® SCE remains fully stable over a wide temperature range, up to 80°C. Nevertheless it is best added during the cooling stage of the formulation.

Microbial activity

Nipaguard® SCE exhibits microbial activity against a wide range of bacteria, yeast and moulds. This is illustrated by the following table which shows the minimum inhibitory concentration (MIC) of Nipaguard® SCE against examples of different groups of microorganisms.

Microorganisms	MIC level [%]
Gram Negative Bacteria	
Pseudomonas aeruginosa	0.4
Escherichia coli	0.4
Gram Positive Bacteria	
Staphylococcus aureus	0.2
Yeasts	
Candida albicans	0.4
Moulds	
Aspergillus brasiliensis	0.4

Storage instructions

The product must be protected from excessively high and low temperatures during storage.

Further information on handling, storage and dispatch is given in the EC safety data sheet.

CLARIANT INTERNATIONAL LTD

Rothausstrasse 61
4132 Muttenz
Switzerland

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www.clariant.com

14.

Spectrastat

Company: Inolex

INCI: Caprylyl Glycol, Caprylhydroxamic Acid, Glycerin

Description

Pale yellowish liquid, compatible with most ingredients. Effective over a pH range from pH 4 - 8 with a typical use level of 0.8 - 1.5% (w/w).

Product Bulletin

Spectrastat™

U.S. Patent No. 8993641

INCI ADOPTED NAME

Caprylyl Glycol (and) Caprylhydroxamic Acid (and) Glycerin

GENERAL INFORMATION

Spectrastat is a blend of multifunctional ingredients that allows formulators to use hurdle technology to create self-preserving formulations. As opposed to traditional methods of preservation, hurdle technology is a formulation approach whereby combinations of ingredients and other aspects of formulation are used along with good manufacturing principles.

Spectrastat includes caprylyl glycol, light, medium spreading emollient, that also have antimicrobial properties, caprylhydroxamic acid (CHA), a chelating agent, and glycerin.

By using **Spectrastat** in the practice of hurdle technology, formulations that pass challenge tests can be created without the inclusion of traditional preservatives such as for example, parabens, isothiazolinones, and formaldehyde donors. These traditional preservatives are currently seen as undesirable by consumers. A special benefit of **Spectrastat** is that it performs superbly at neutral pH, a state where many other alternative preservation systems are ineffective

Spectrastat 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 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.

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 0.7% w/w to 1.2% w/w.

PHYSICAL PROPERTIES (TYPICAL)

Appearance..... Pale yellow liquid, 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**. Refer to the material Safety Data Sheet for (SDS) further information.

SAFETY DATA

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

STANDARD PACKAGING

Plastic pail, 55 lb (24.95 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

15.

TRIstat E

Company: TRI-K

INCI: Benzyl alcohol, Potassium sorbate, Sodium Benzoate, Aqua

Description

Broad spectrum, active at pH under 5.5, ECOCERT, COSMOS approved

TRI-K
A MEMBER OF THE Galaxy GROUP

TRIstat E

MODERN PRESERVATIVE BASED ON NATURE-IDENTICAL INGREDIENTS

INCI: Benzyl Alcohol, Potassium Sorbate, Sodium Benzoate, Water

BENEFITS

Broad spectrum preservative system

Active at pH <5.5

Gentle to the skin

Soluble in glycerin, glycols & water

FEATURES

Based on nature-identical ingredients

Free from parabens, formaldehyde donors & isothiazolinones

Permitted in ECOCERT/COSMOS formulations

Cost effective modern system

APPLICATIONS

Natural & organic formulations

Aqueous gels & serums

Surfactant based formulations

Face & body creams and lotions

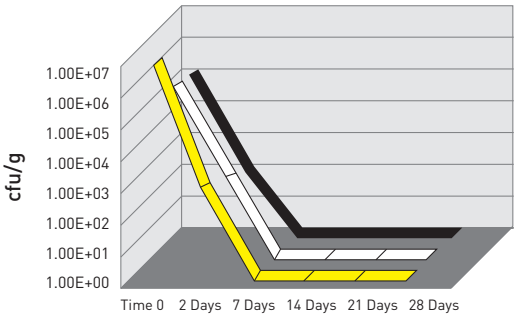
Soluble in select oil based formulation

FORTIFY • PROTECT • UNIQUE

TRIstat E

MODERN PRESERVATIVE BASED ON NATURE-IDENTICAL INGREDIENTS

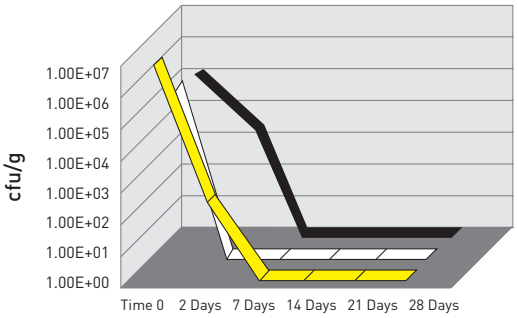
PRESERVATION EFFICACY IN OIL/WATER EMULSION, pH 5.5 WITH 1.2% TRISTAT E



1.2% TRIstat E has shown to be effective in preserving the tested formula in 7 days

STRAINS	0 DAYS	2 DAYS	7 DAYS	14 DAYS	21 DAYS	28 DAYS	RESULTS
○ E. coli	$7.7 \cdot 10^6$	$7.5 \cdot 10^2$	<10	<10	<10	<10	Passed
○ P. aeruginosa	$7.7 \cdot 10^6$	$7.5 \cdot 10^2$	<10	<10	<10	<10	Passed
○ S. aureus	$7.7 \cdot 10^6$	$7.5 \cdot 10^2$	<10	<10	<10	<10	Passed
○ C. albicans	$2.5 \cdot 10^5$	$4.2 \cdot 10^2$	<10	<10	<10	<10	Passed
● A. niger	$1.4 \cdot 10^5$	$1.6 \cdot 10^2$	<10	<10	<10	<10	Passed

PRESERVATION EFFICACY IN PEG & SULFATE-FREE SHOWER BATH, pH 5.0 WITH 0.7% TRISTAT E



0.7% TRIstat E has shown to be effective in preserving the tested formula in 7 days

STRAINS	0 DAYS	2 DAYS	7 DAYS	14 DAYS	21 DAYS	28 DAYS	RESULTS
○ E. coli	$7.7 \cdot 10^6$	$2.7 \cdot 10^2$	<10	<10	<10	<10	Passed
○ P. aeruginosa	$7.7 \cdot 10^6$	$2.7 \cdot 10^2$	<10	<10	<10	<10	Passed
○ S. aureus	$7.7 \cdot 10^6$	$2.7 \cdot 10^2$	<10	<10	<10	<10	Passed
○ C. albicans	$2.5 \cdot 10^5$	<10	<10	<10	<10	<10	Passed
● A. niger	$1.4 \cdot 10^5$	$2.7 \cdot 10^3$	<10	<10	<10	<10	Passed

EFFICACY TESTS

The antimicrobial activity of **TRIstat E** in different cosmetic formulations was evaluated by challenge testing, using a modified European Pharmacopoeia method

INHIBITORY (MIC) & BIOCIDAL (MBC) ACTIVITY OF TRISTAT E IN PPM

TEST ORGANISMS (≈10^6CFU/ML)	MBC	MIC
Gram-negative bacterium *Escherichia coli*	5000	2000
Gram-negative bacterium *Pseudomonas aeruginosa*	5000	2500
Gram-positive bacterium *Staphylococcus aureus*	4000	1750
Yeast *Candida albicans*	3500	3000
Mold *Aspergillus niger*	6000	4000

TRIstat E has **bacteriostatic & fungistatic effect** at levels of 0.4%. It exhibits bactericidal & fungicidal activity at 0.6%

PROPERTIES

TRIstat E is a unique and very effective preservation system suitable for the protection of cosmetics against microbial contamination

FORTIFY • PROTECT • UNIQUE

TRI-K Industries, Inc.
p +1 (800) 526-0372
e info@tri-k.com
www.tri-k.com

TRI-K
A MEMBER OF THE Galaxy GROUP

SECTION 1: IDENTIFICATION OF THE SUBSTANCE/MIXTURE AND THE COMPANY/UNDERTAKING

1.1 Product Identifier:

Trade Name	TRIstat E
INCI Name	Benzyl alcohol, Water, Potassium sorbate, Sodium benzoate

1.2 Relevant Identified Uses of the Substance or Mixture and Uses Advised Against:

Product Use:	Active ingredient in cosmetic and personal care applications

1.3 Details of the Supplier of the Safety Data Sheet:

Manufacturer:	TRI-K INDUSTRIES, INC. 2 Stewart Court Denville, NJ 07834
Information Phone Number:	(973) 298-8850
E-mail	info@tri-k.com

1.4 Emergency Telephone Number:

Emergency Spill Information	(973) 298-8850 (TRI-K Industries, Inc.) (800) 222-1222 (National Poison Control Center)
24-HOUR EMERGENCY TELEPHONE NUMBER	**CHEMTREC +1 (800) 424-9300 or +1 (703) 527-3887**

SDS Date of Preparation: July 29, 2015
SDS Date of Preparation: June 23, 2017

SECTION 2: HAZARDS IDENTIFICATION

2.1. Classification of the substance or mixture.

The product is classified as hazardous pursuant to the provisions set forth in EC Regulation 1272/2008 (CLP) (and subsequent amendments and supplements). The product thus requires a safety datasheet that complies with the provisions of EC Regulation 1907/2006 and subsequent amendments.

Any additional information concerning the risks for health and/or the environment are given in sections 11 and 12 of this sheet.

Hazard classification and indication: Acute toxicity, category 4 H302 Harmful if swallowed. Eye irritation, category 2 H319 Causes serious eye irritation.

2.2. Label elements.

Hazard labelling pursuant to EC Regulation 1272/2008 (CLP) and subsequent amendments and supplements.

Signal words: Warning

Hazard statements:
H302 Harmful if swallowed.
Precautionary statements:
P280 Wear eye protection / face protection. P301+P312 IF SWALLOWED: call a POISON CENTER / doctor if you feel unwell. P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P337+P313 If eye irritation persists: Get medical advice / attention. Contains: Benzyl alcohol

SECTION 3: COMPOSITION/INFORMATION ON INGREDIENTS

The full wording of hazard (H) phrases is given in section 16 of the sheet.

Identification.	**Conc. %.**	
Benzyl alcohol		
CAS. 100-51-6	52	Acute Tox. 4 H302, Acute Tox. 4 H332, Eye Irrit. 2 H319
EC. 202-859-9		
INDEX. 603-057-00-5		
Reg. no. 01-2119492630-38		
Potassium (E,E)-hexa-2,4-dienoate		
CAS. 24634-61-5	16	Eye Irrit. 2 H319
EC. 246-376-1		
INDEX. -		
Sodium Benzoate		
CAS. 532-32-1	11	Eye Irrit. 2 H319
EC. 208-534-8		
INDEX. -		
Reg. no. 01-2119460683-35		

Additional information: For full text of H-statements and R-phrases: see SECTION 16

SECTION 4: FIRST AID MEASURES

4.1 Description of First Aid Measures:

EYES: Remove contact lenses, if present. Wash immediately with plenty of water for at least 15 minutes, opening the eyelids fully. If problem persists, seek medical advice.
SKIN: Remove contaminated clothing. Wash immediately with plenty of water. If

irritation persists, get medical advice/attention. Wash contaminated clothing before using it again.

INHALATION: Remove to open air. In the event of breathing difficulties, get medical advice/attention immediately.

INGESTION: Get medical advice/attention. Induce vomiting only if indicated by the doctor. Never give anything by mouth to an unconscious person, unless authorized by a doctor.

4.2 Most Important symptoms and effects, both acute and delayed:

Not expected to be a skin or eye irritant (based on available data). Non-toxic by oral ingestion (based on nature of material). No other adverse clinical effects are known to be associated with exposure to this material.

4.3 Indication of any immediate medical attention and special treatment needed:

No immediate medical treatment normally needed.

SECTION 5: FIRE FIGHTING MEASURES

5.1 Suitable Extinguishing Media:

SUITABLE EXTINGUISHING EQUIPMENT

The extinguishing equipment should be of the conventional kind: carbon dioxide, foam, powder and water spray.

UNSUITABLE EXTINGUISHING EQUIPMENT

None in particular.

5.2 Special Hazards Arising from the Substance or Mixture:

Unusual Fire and Explosion Hazards: None known

Hazardous Decomposition Products: None known

HAZARDS CAUSED BY EXPOSURE IN THE EVENT OF FIRE

Do not breathe combustion products.

5.3 Advice for Fire-Fighters:

Special Fire Fighting Procedures:

Use jets of water to cool the containers to prevent product decomposition and the development of substances potentially hazardous for health. Always wear full fire prevention gear. Collect extinguishing water to prevent it from draining into the sewer system. Dispose of contaminated water used for extinction and the remains of the fire according to applicable regulations.

SPECIAL PROTECTIVE EQUIPMENT FOR FIRE-FIGHTERS

Normal firefighting clothing i.e. fire kit (BS EN 469), gloves (BS EN 659) and boots (HO specification A29 and A30) in combination with self-contained open circuit positive pressure compressed air breathing apparatus (BS EN 137).

SECTION 6: ACCIDENTAL RELEASE MEASURES

6.1 Personal Precautions, Protective Equipment and Emergency Procedures:

Block the leakage if there is no hazard.

Wear suitable protective equipment (including personal protective equipment referred to under Section 8 of the safety data sheet) to prevent any contamination of skin, eyes and personal clothing. These indications apply for both processing staff and those involved in emergency procedures.

6.2 Environmental Precautions:

The product must not penetrate into the sewer system or come into contact with surface water or ground water.

6.3 Methods and Material for Containment and Cleaning Up:

Collect the leaked product into a suitable container. Evaluate the compatibility of the container to be used, by checking section 10. Absorb the remainder with inert absorbent material.

Make sure the leakage site is well aired. Check incompatibility for container material in section 7. Contaminated material should be disposed of in compliance with the provisions set forth in point 13.

6.4 Reference to Other Sections:

Refer to Section 8 for protective equipment and Section 13 for disposal considerations

SECTION 7: HANDLING AND STORAGE

7.1 Precautions for Safe Handling:

Before handling the product, consult all the other sections of this material safety data sheet. Avoid leakage of the product into the environment. Do not eat, drink or smoke during use. Remove any contaminated clothes and personal protective equipment before entering places in which people eat.

7.2 Conditions for Safe Storage, Including any Incompatibilities:

Store at room temperature in tightly sealed containers. Avoid temperatures above 40°C as this may affect the efficacy of the product. Optimum storage temperature is 24°C or lower. Do not freeze. Avoid exposure to sunlight for prolonged periods.

7.3 Specific end use(s): Active ingredient in cosmetic and personal care applications

SECTION 8: EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 Control Parameters:

Sodium Benzoate

Health - Derived no-effect level - DNEL / DMEL

	Effects on consumers.				Effects on workers		
Route of exposure	Acute local	Acute systemic	Chronic local	Chronic systemic	Acute local	Acute systemic	Chronic local
Oral				25 mg/kg/d			
VND							
Inhalation.		1,3 mg/m3		2,1 mg/m3		6,3 mg/m3	10,4
Skin.		2,7 mg/cm2		VND		4,5 mg/cm2	VND

Benzyl alcohol

Predicted no-effect concentration - PNEC.

Normal value in fresh water	1	mg/l
Normal value for marine water	0,527	mg/l
sediment		
Normal value for water, intermittent release	2,3	mg/l
Normal value of STP microorganisms	39	mg/l
Normal value for the terrestrial compartment	0,456	mg/kg

Health - Derived no-effect level - DNEL / DMEL

Effects on consumers.					Effects on workers		
Route of exposure	Acute local	Acute systemic	Chronic local	Chronic systemic	Acute local	Acute systemic	Chronic local
Oral	VND	25 mg/kg	VND	5 mg/kg			
Inhalation	VND	95,5 mg/m3	VND	19,1 mg/m3	VND	450 mg/m3	VND
Skin.	VND	28,5 mg/kg	VND	5,7 mg/kg	VND	47 mg/kg	VND

8.2 Exposure Controls:

As the use of adequate technical equipment must always take priority over personal protective equipment, make sure that the workplace is well aired through effective local aspiration.

When choosing personal protective equipment, ask your chemical substance supplier for advice. Personal protective equipment must be CE marked, showing that it complies with applicable

standards.
Provide an emergency shower with face and eye wash station.

HAND PROTECTION

Protect hands with category III work gloves.
The following should be considered when choosing work glove material: compatibility, degradation, failure time and permeability. The work gloves' resistance to chemical agents should be checked before use, as it can be unpredictable. The gloves' wear time depends on the duration and type of use.

SKIN PROTECTION

Wear category I professional long-sleeved overalls and safety footwear (see Directive 89/686/EEC and standard EN ISO 20344). Wash body with soap and water after removing protective clothing.

EYE PROTECTION

Wear airtight protective goggles (see standard EN 166).
In the presence of risks of exposure to splashes or squirts during work, adequate mouth, nose and eye protection should be used to prevent accidental absorption.

RESPIRATORY PROTECTION

If the threshold value (e.g. TLV-TWA) is exceeded for the substance or one of the substances present in the product, use a mask with a type A filter whose class (1, 2 or 3) must be chosen according to the limit of use concentration. (see standard EN 14387). In the presence of gases or vapours of various kinds and/or gases or vapours containing particulate (aerosol sprays, fumes, mists, etc.) combined filters are required.
Respiratory protection devices must be used if the technical measures adopted are not suitable for restricting the worker's exposure to the threshold values considered. The protection provided by masks is in any case limited.
If the substance considered is odourless or its olfactory threshold is higher than the corresponding TLV-TWA and in the case of an emergency, wear open-circuit compressed air breathing apparatus (in compliance with standard EN 137) or external air-intake breathing apparatus (in compliance with standard EN 138). For a correct choice of respiratory protection device, see standard EN 529.

ENVIRONMENTAL EXPOSURE CONTROLS.

The emissions generated by manufacturing processes, including those generated by ventilation equipment, should be checked to ensure compliance with environmental standards.

SECTION 9: PHYSICAL AND CHEMICAL PROPERTIES

9.1 Information on basic Physical and Chemical Properties:

Appearance: Liquid, Yellow	**Vapor Pressure:** Not determined
Odor: Characteristic	**Vapor Density:** Not determined
Odor Threshold: No data available	**Specific Gravity:** Not Determined
pH: 8	**Water Solubility:** Soluble
Melting Point / freezing point: Not determined	**Partition coefficient: n-octanol/water:** Not Available
Boiling Point: Not applicable	**Auto-ignition Temperature:** Not Available
Flash Point (COC): > 60 °C.	**Decomposition Temperature:** Not Available
Evaporation Rate: Not Applicable	**Viscosity (cP):** Not determined
Flammability (solid, gas): Not Applicable	**Explosion Properties:** Not determined
Upper/lower flammability or explosive limits: Not Applicable	**Oxidizing Properties:** None

9.2 Other Information:

None

SECTION 10: STABILITY AND REACTIVITY

10.1 Reactivity:

There are no particular risks of reaction with other substances in normal conditions of use.

10.2 Chemical Stability:

The product is stable in normal conditions of use and storage

10.3 Possibility of Hazardous Reactions:

No hazardous reactions are foreseeable in normal conditions of use and storage

10.4 Conditions to Avoid:

Gross bacterial contamination and Heat

10.5 Incompatible Materials:

Oxidant agents.

10.6 Hazardous Decomposition Products:

Burning can produce smoke, CO, CO_2, ammonia and other products of incomplete combustion

SECTION 11: TOXICOLOGICAL INFORMATION

11.1 Information on Toxicological Effects:

In the absence of experimental data for the product itself, health hazards are evaluated according to the properties of the substances it contains, using the criteria specified in the applicable regulation for classification.It is therefore necessary to take into account the concentration of the individual hazardous substances indicated in section 3, to evaluate the toxicological effects of exposure to the product.
Acute effects: ingestion of this product is harmful. Even small amounts of product may cause serious health problems (stomach pain, nausea, sickness, diarrhoea).
Acute effects: stinging eyes. Symptoms may include: rubescence, edema, pain and lachrymation.
Ingestion may cause health problems, including stomach pain and sting, nausea and sickness.
Benzyl alcohol
Acute oral toxicity (rat) LD50 = 1230 mg/kg
Acute dermal toxicity (rabbit) LD50 = 2000 mg/kg
Acute inhalation toxicity (rat) LC50 > 500 mg/m3/4h
Subchronic oral toxicity (rat) NOAEL = 400 mg/kg/day (90 days)
Skin irritation (rabbit) Not irritating
Eye irritation (rabbit) Irritating
Sensitization (guinea pig) Not sensitizing
Chronical toxicity No carcinogenic, mutagenic or teratogenic effect known.

SECTION 12: ECOLOGICAL INFORMATION

12.1 Toxicity:

Benzyl alcohol

LC50 - for Fish.	460 mg/l/96h Pimephales promelas
EC50 - for Crustacea.	230 mg/l/48h Daphnia magna
EC50 - for Algae / Aquatic Plants.	310 mg/l/72h Pseudokirchneriella subcapitata

Sodium Benzoate

LC50 - for Fish.	> 100 mg/l/96h OECD 203
EC50 - for Crustacea.	> 100 mg/l/48h

Chronic NOEC for Crustacea.	Daphnia magna 51 mg/l/21d Daphnia magna

Potassium (E,E)-hexa-2,4-dienoate

LC50 - for Fish.	1250 mg/l/96h Brachydanio rerio
EC50 - for Crustacea.	982 mg/l/48h Daphnia magna

12.2 Persistence and Degradability:

Benzyl alcohol
Rapidly biodegradable.
Sodium Benzoate Solubility in water. 556 g/l 20°C
Rapidly biodegradable.
Potassium (E,E)-hexa-2,4-dienoate
Rapidly biodegradable.

12.3 Bio accumulative Potential:

Benzyl alcohol

Partition coefficient: n-octanol/water.	1,05
BCF.	1,37

Sodium Benzoate

Partition coefficient: n-octanol/water.	-2,27

Potassium (E,E)-hexa-2,4-dienoate
Partition coefficient: n-octanol/water. -1,72

12.4 Mobility in Soil:

Since the product is completely soluble in water, it is expected to be highly mobile in soil.

12.5 Results of PBT and vPvB Assessment:

This mixture does not contain any substances that are assessed to be a PBT or a vPvB.

12.6 Other Adverse Effects:

Information Not Available

SECTION 13: DISPOSAL CONSIDERATIONS

13.1 Waste Treatment Methods:

Reuse, when possible. Product residues should be considered special hazardous waste. The hazard level of waste containing this product should be evaluated according to applicable regulations.

Disposal must be performed through an authorised waste management firm, in compliance with national and local regulations.

CONTAMINATED PACKAGING

Contaminated packaging must be recovered or disposed of in compliance with national waste management regulations.

SECTION 14: TRANSPORT INFORMATION

	US DOT	EU land transport (ADR/RID/ADN)	Sea Transport (IMDG)	Air Transport (ICAO/IATA)
14.1 UN Number				
14.2 UN Proper Shipping Name	Not regulated	Not regulated	Not regulated	Not regulated
14.3 Transport Hazard Class(s)				
14.4 Packing Group				
14.5 Environmental Hazards	Not applicable	Not applicable	Not applicable	Not applicable
14.6 Special Precautions for user	None	None	None	None
14.7 Transport in bulk according to Annex II of MARPOL 73/78 and the IBC Code	Not applicable	Not applicable	Not applicable	Not applicable

SECTION 15: REGULATORY INFORMATION

15.1 Safety, Health and Environment Regulations/Legislation Specific for the Substance or Mixture:

EU EINECS/ELINCS/NLP:	All of the components of this product are listed on the EINECS Inventory.
Canada DSL/NDSL:	All of the components of this product are listed on the DSL.
US TSCA	All of the components of this product are listed on the US TSCA.

China IECSC:	All of the components of this product are listed on the IECSC.
Japan ENCS:	All of the components are listed on the Japanese Existing and New Chemical Substances Inventory.
Philippine PICCS:	All of the components of this product are listed on the PICCS.
Australia AICS:	All of the components of this product are listed on the AICS.

15.2 Chemical Safety Assessment:
Not required

SECTION 16: OTHER INFORMATION

16.1 Indication of Changes
Version 1 created on July 29, 2015
Version 1 created on June 23, 2017

16.2 List of Relevant R- phrases (number and full text):
Not applicable

16.3 Legal Disclaimer

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16.

Sorbic Acid and Potassium Sorbate as Cosmetic Preservatives

Article by Eastman

Eastman

Sorbic Acid and Potassium Sorbate as Cosmetic Preservatives

eastman

Contents

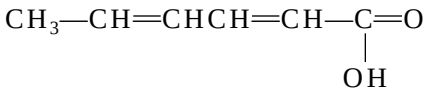
Key Characteristics

- 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

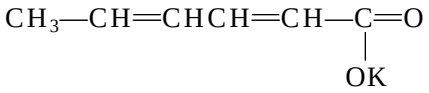
Sorbic acid and potassium sorbate are excellent, safe preservatives for cosmetics and personal care products with a pH lower than 6.5. They have good skin compatibility and are easy to use, especially potassium sorbate in salt form.

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

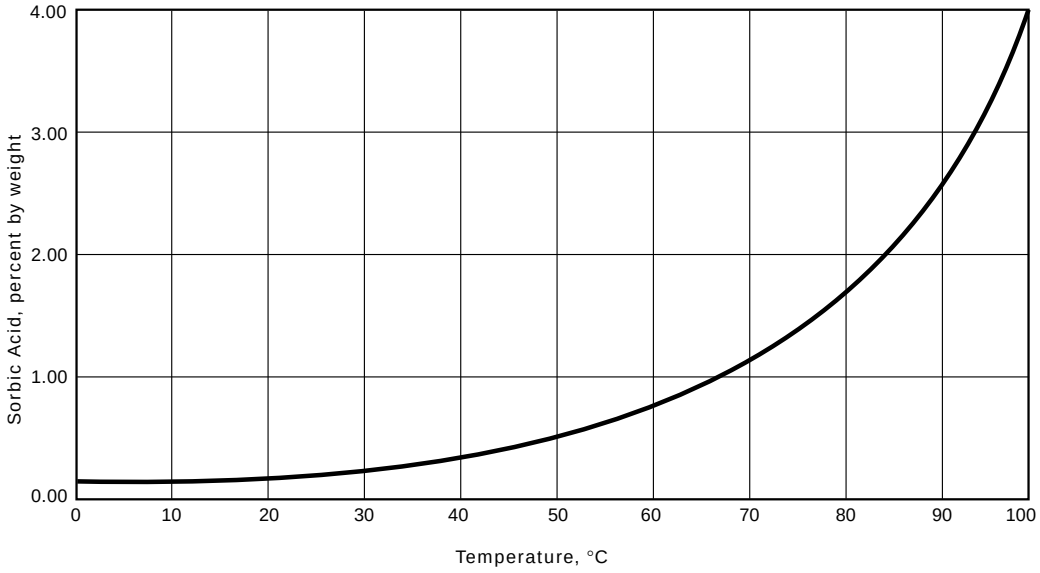
[a]*Properties reported here are typical of average lots. Eastman makes no representation that the material in any shipment will conform to the values given.*
[b]*International Nomenclature Cosmetic Ingredient; Cosmetic, Toiletry, and Fragrance Association.*

NA—*Not Applicable*

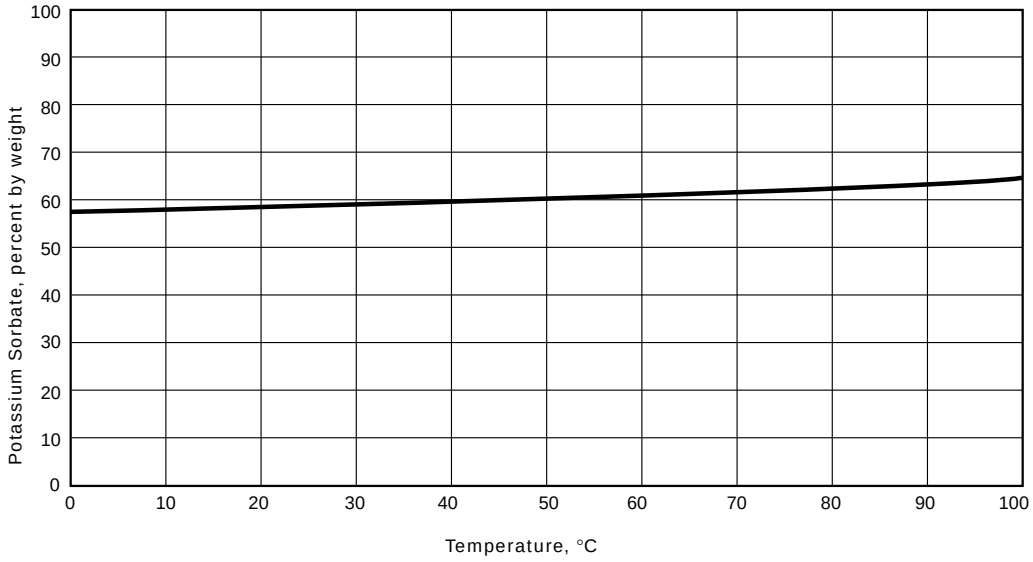
Eastman sorbic acid and *Eastman* potassium sorbate are highly refined, white to off-white, free-flowing powders or granules. Sorbic acid provides greater antimicrobial potency than potassium sorbate. However, in water, sorbic acid is barely soluble while potassium sorbate is extremely soluble. Therefore, 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

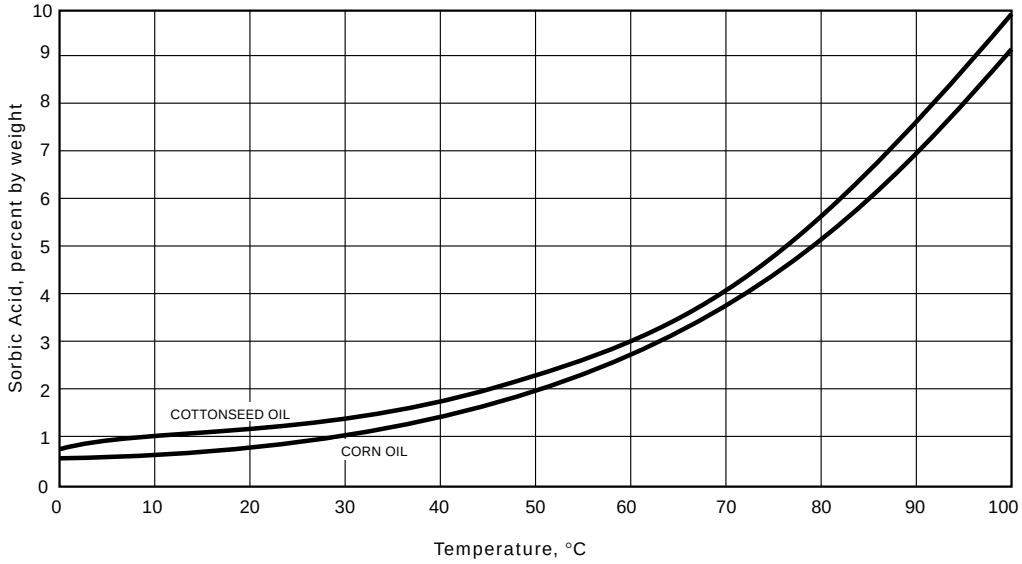


POTASSIUM SORBATE, 0° TO 100°C

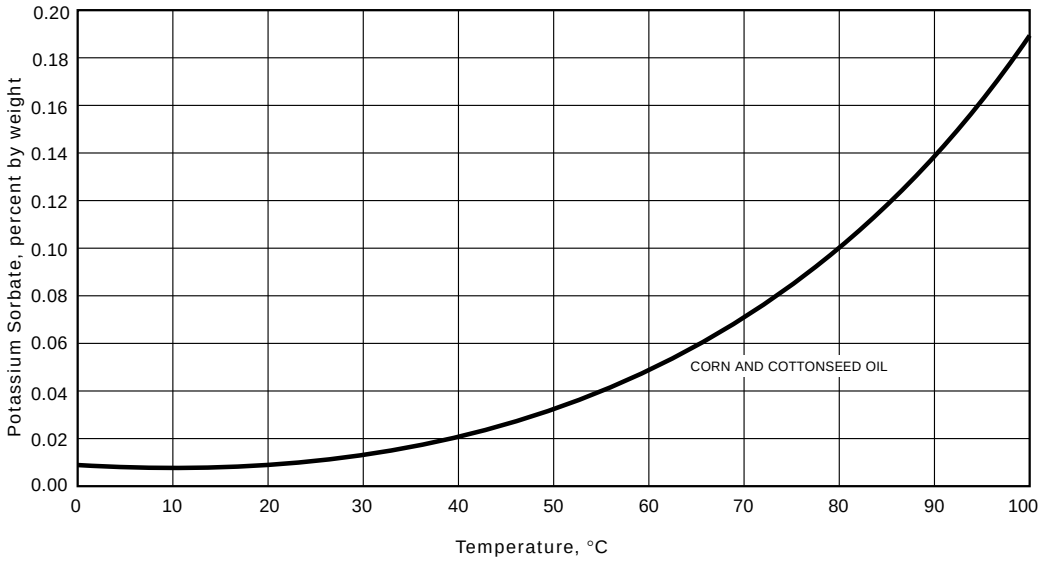


Solubility in Corn and Cottonseed Oils

SORBIC ACID, 0° TO 100°C

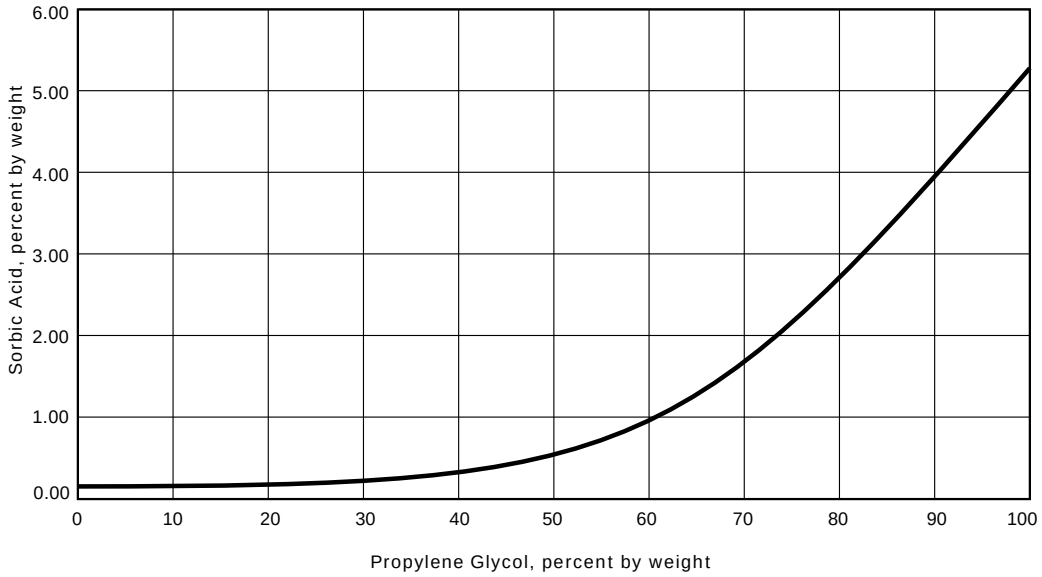


POTASSIUM SORBATE, 0° TO 100°C

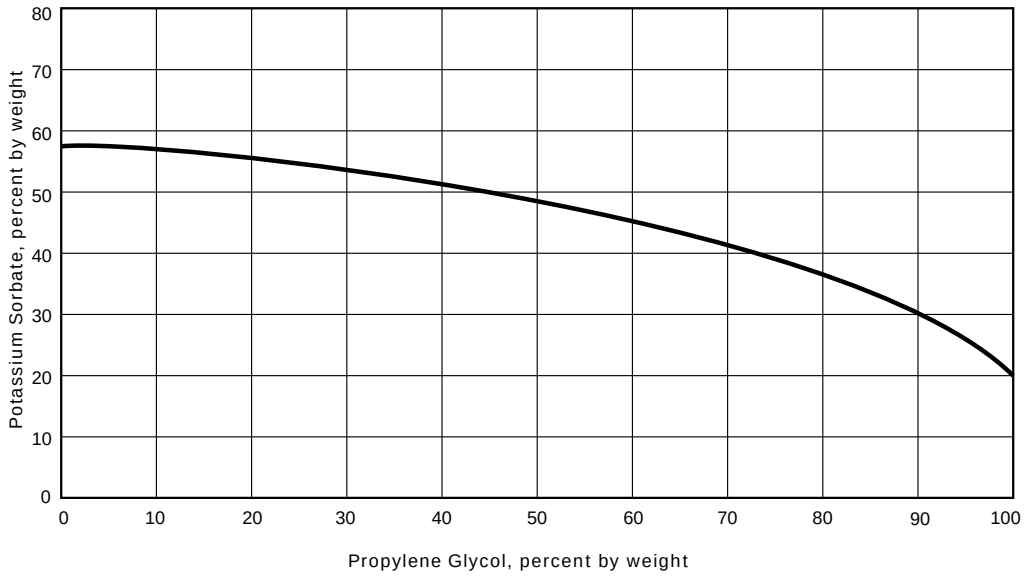


Solubility in Propylene Glycol/ Water Solutions

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

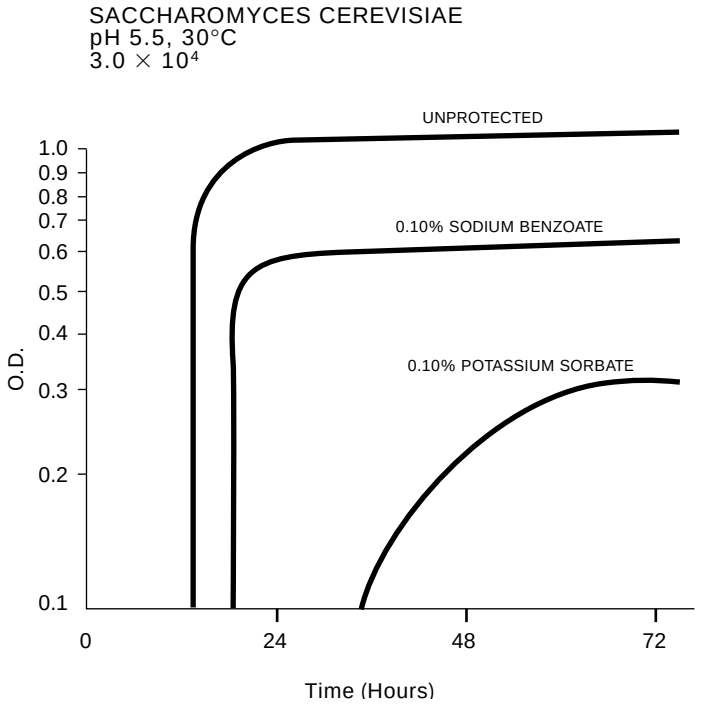
Acetobacter aceti[c]
Acetobacter xylinum[c]
Achromobacter sp.[c]
Alcaligenes faecalis[c]
Azotobacter agilis[c]
Bacillus coagulans[c]
Bacillus cereus[c]
Bacillus poymyxa[c]
Bacillus stearothermophilus[c]
Bacillus subtilis[c]
Clostridium perfringens[a]
Clostridium sporogenes[a]
Clostridium tetani[d]
Enterobacter 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

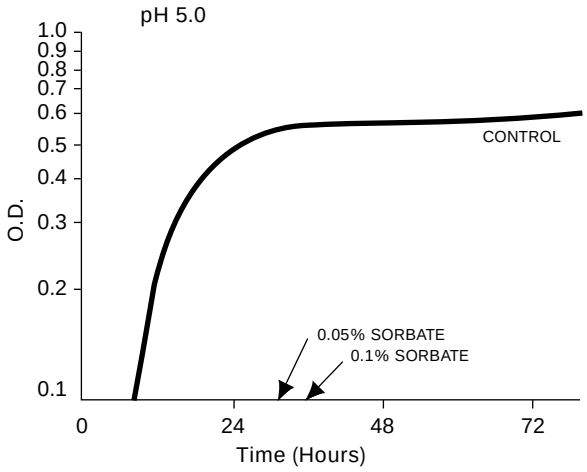
The antimicrobial potency of all commercial cosmetic preservatives is pH-dependent. Sorbates are more effective at higher pH ranges than other organic acids used as preservatives. Sorbates are effective up to 6.5, whereas benzoates are effective to only 4.5. These preservative compounds can be used in 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.

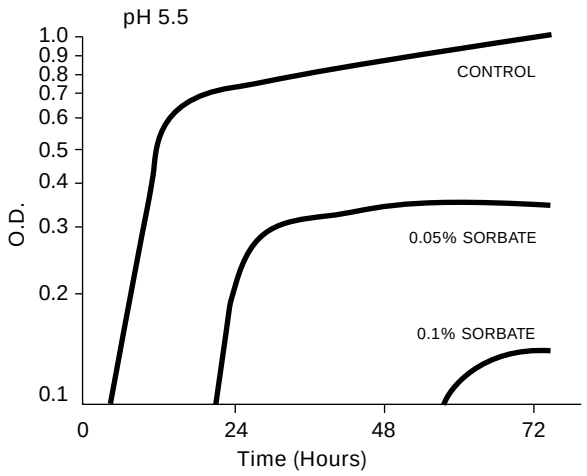
The graph shows the relative inhibition of yeast by equal concentrations of sorbate and benzoate at pH 5.5 and 30°C when a broth is inoculated with 3×10^4 organisms / mL. Growth is measured by the optical density of the broth. Sorbate significantly delays growth, and the amount of ultimate growth at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

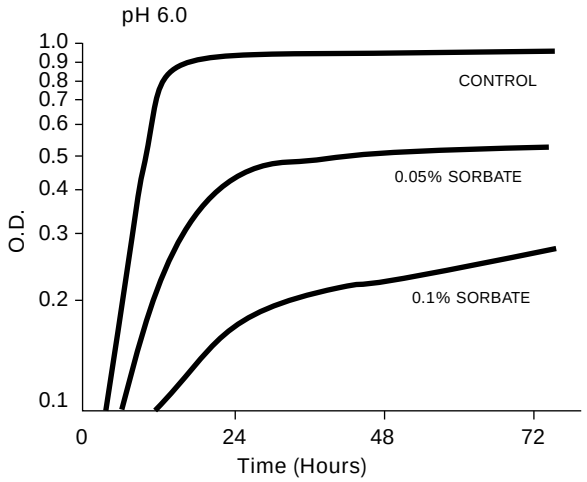


The following graphs show the effectiveness of sorbate at pH 5.0, 5.5, 6.0, and 6.5

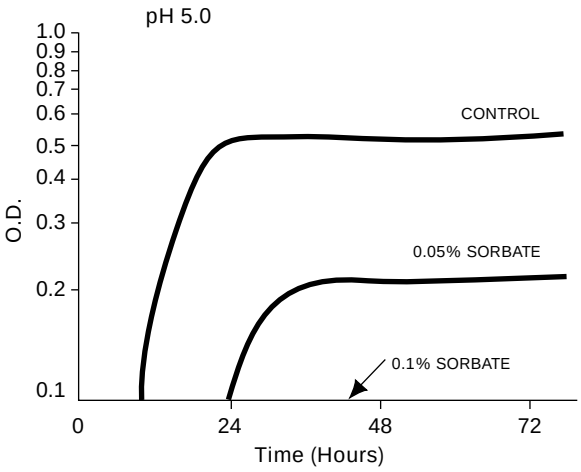
ESCHERICHIA COLI

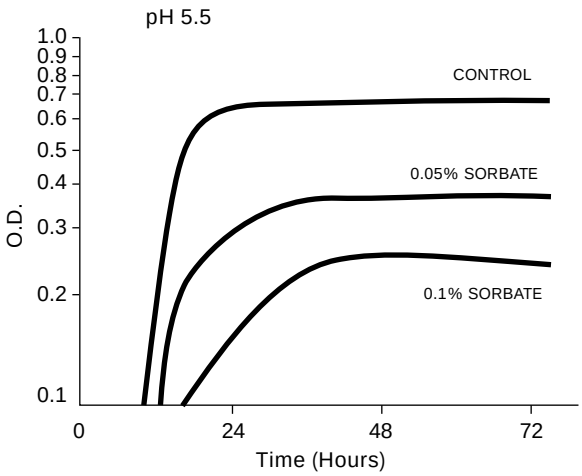


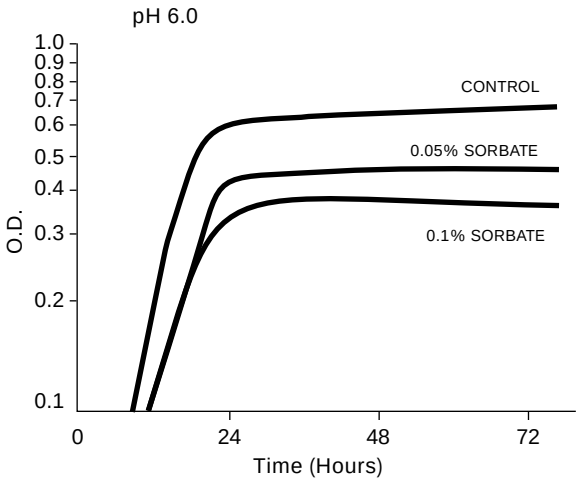




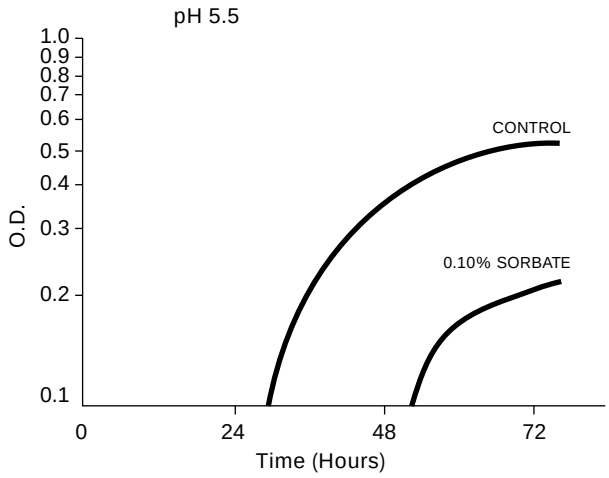
SACCHAROMYCES CEREVISIAE

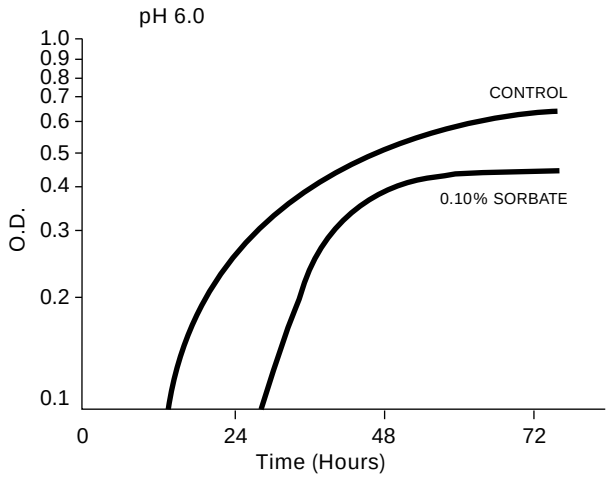


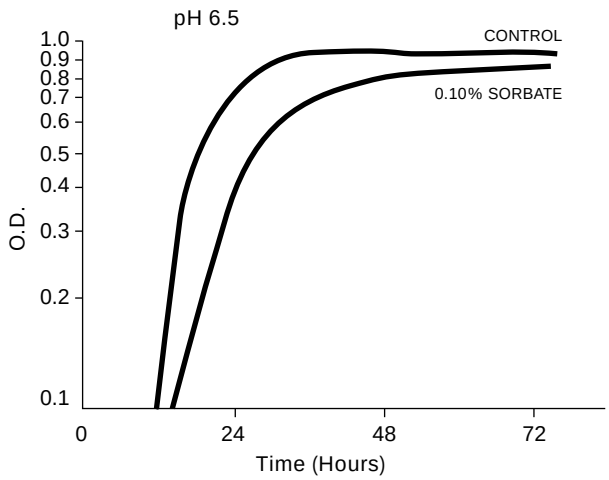




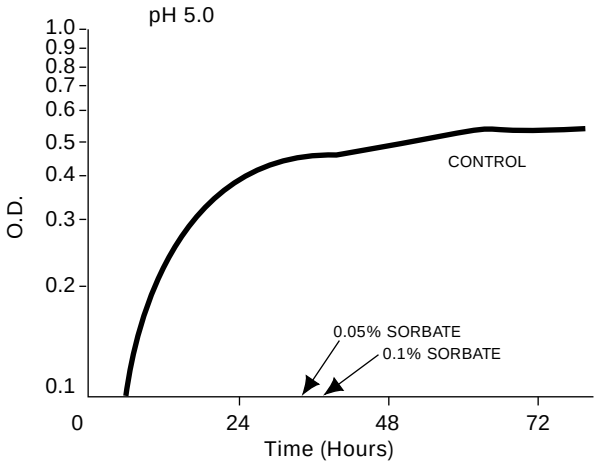
STAPHYLOCOCCUS AUREUS

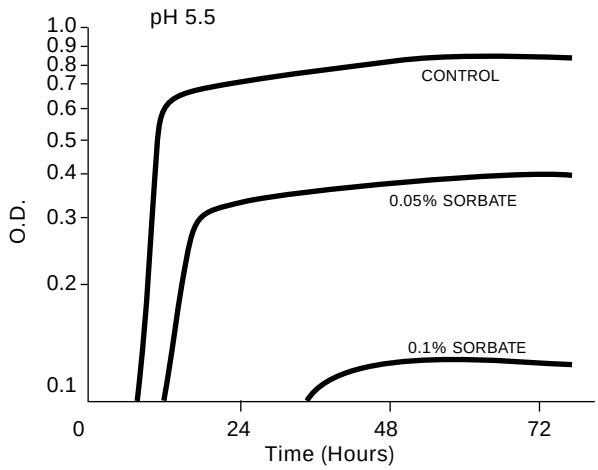


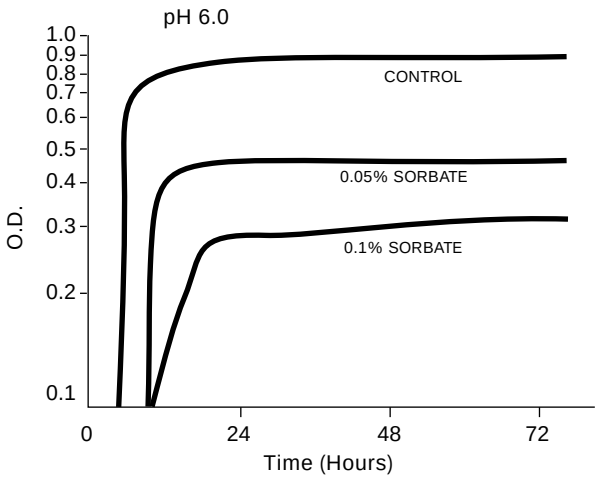




SALMONELLA







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

NORTH AMERICA

Eastman Chemical Company
Corporate Headquarters
P.O. Box 431
Kingsport, TN 37662-5280 U.S.A.

Telephone:
U.S.A. and Canada, 800-EASTMAN (800-327-8626)
Other Locations (1) 423-229-2000
Fax: (1) 423-229-1673

http://www.eastman.com

LATIN AMERICA

Eastman Chemical Latin America, Inc.
2333 Ponce de Leon Blvd.
Suite R-20
Coral Gables, FL 33134 U.S.A.

Telephone: (1) 305-461-8240
Fax: (1) 305-461-8254

EUROPE / MIDDLE EAST / AFRICA

Eastman Chemical, Europe, Middle East, and Africa Ltd.
Tobias Asserlaan 5
2517 KC The Hague
NETHERLANDS

Telephone: (31) 70 370 1711
Fax: (31) 70 370 1704

ASIA PACIFIC

Eastman Chemical Japan Ltd.
Yebisu Garden Place Tower, 32F
4-20-3 Ebisu
Shibuya-ku, Tokyo 150 JAPAN

Telephone: (81) 3-5424-1551
Fax: (81) 3-5424-1590

Eastman Chemical Asia Pacific Pte. Ltd.
#05-04 Winsland House
3 Killiney Road
Singapore 239519 SINGAPORE

Telephone: (65) 738-4877
Fax: (65) 732-4930

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17.

Weak-Acid Preservatives

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Weak-acid preservatives: modelling microbial inhibition and response

R.J. Lambert and M. Stratford

Microbiology Section, Unilever Research, Sharnbrook, Bedford UK

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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

Correspondence to: Dr R.J. Lambert, Microbiology Section, Unilever Research, Colworth House, Sharnbrook, Bedford MK44 1LQ, UK (e-mail: RONNIE.LAMBERT@UNILEVER.COM).

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.

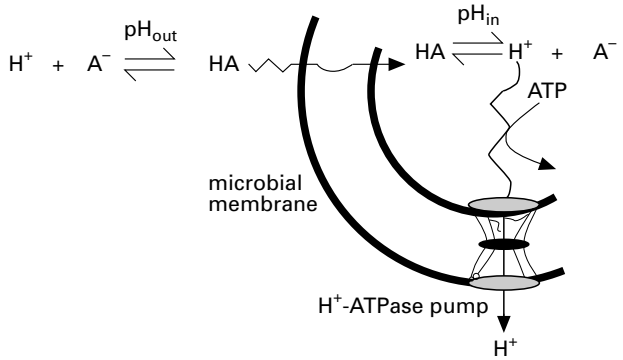

Fig. 1 Predicted medium and cytoplasmic weak-acid/anion equilibria. Only uncharged weak-acid molecules (HA) can diffuse freely across the plasma membrane. Charged anions (A^-) and protons (H^+) are retained within the cell; cytoplasmic protons are expelled by the membrane-bound H^+-ATPase

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}

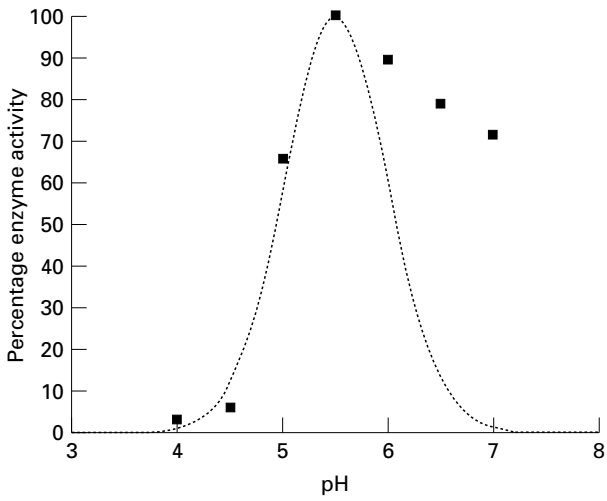

Fig. 2 Gaussian fit of the pH profile of the plasma-membrane H^+-ATPase (-----), based on the experimental data (■) of Willsky (1979)

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

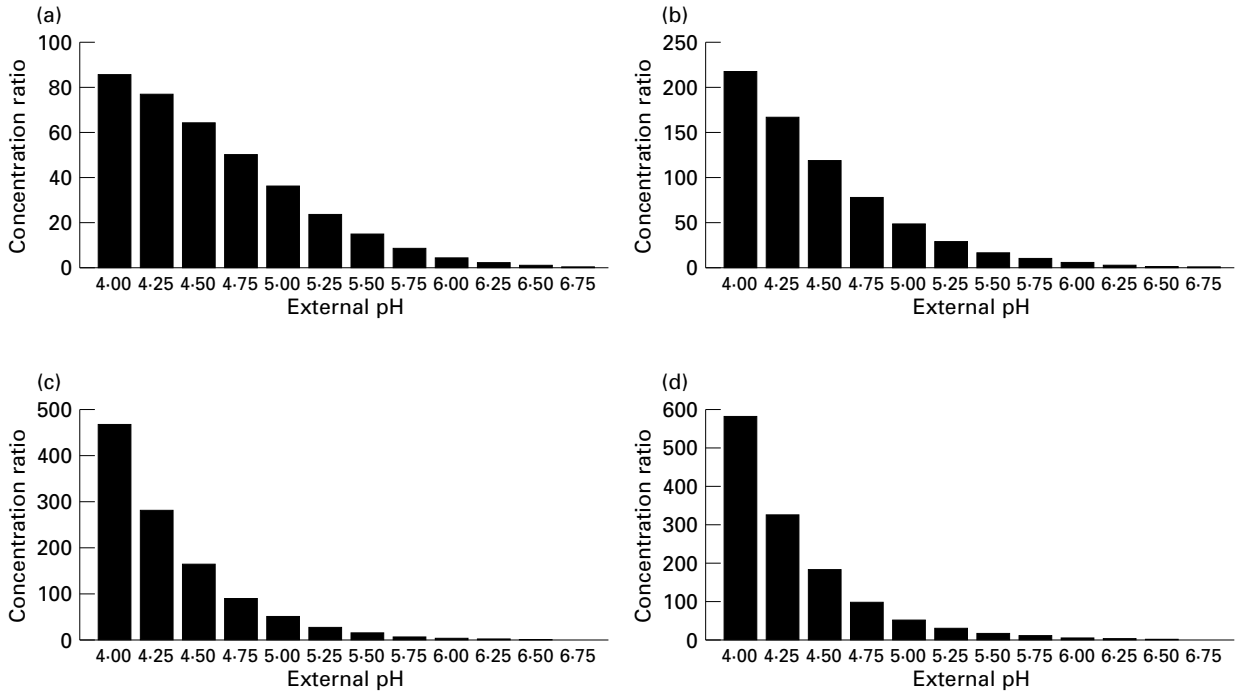

Fig. 3 Predicted concentration ratios of preservatives from medium to cytoplasm, based on known proportions of undissociated acid/anion at each pH value (Table 1). Concentrations are calculated assuming pH_i to be 6·75, due either to infinite buffering or to proton removal. (a) Sorbic acid/sorbate; (b)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

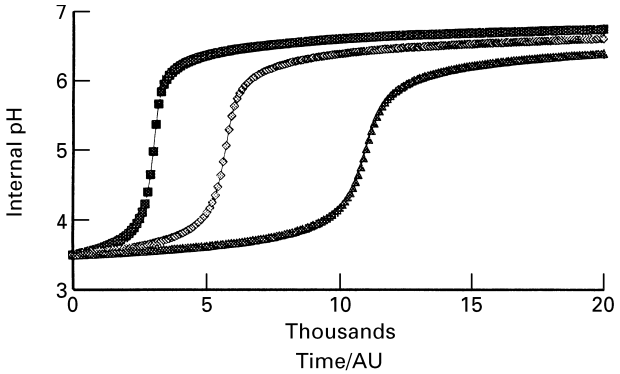

Fig. 4 Modelling the rise of pH_i from pH 3·5 by proton pumping, despite further weak-acid influx. Sorbic acid concentrations used were 0·5 mmol l^{-1} (■), 1 mmol l^{-1} (◇) and 2 mmol l^{-1} (▲). Time is in arbitrary units. Increased time is required (lag phase) to raise pH_i with increased preservative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

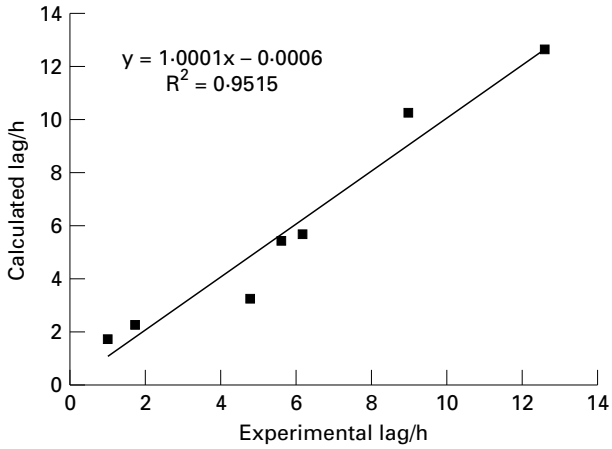

Fig. 5 Scatter plot of calculated and experimentally-determined lag phases of *Saccharomyces cerevisiae* X2180–1B

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).

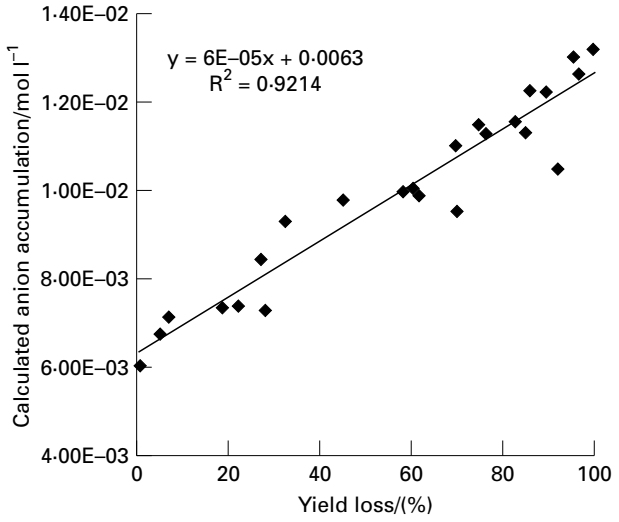

Fig. 6 Scatter plot of experimentally-determined loss of cell yield of *Saccharomyces cerevisiae* X2180–1B against calculated accumulation of anion. It is predicted that each anion accumulated represents expenditure of one ATP in proton extrusion. Hence, calculated ATP usage shows a linear relationship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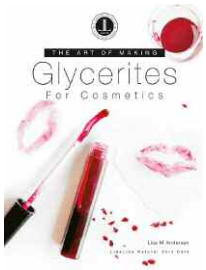


The Art of Making Glycerites for Cosmetics

Learn how to make your own glycerine extracts with herbs, flowers, and foods – fresh, dried, freeze-dried, and semi-dried. This book includes calculation charts and guides for making both self-preserving glycerites and extracts with added preservatives. Includes 6 unique cosmetic formulas featuring handcrafted glycerites - from shower gel to face cream.

114 page e-book

Guide to Hiring a Formulator

This handy guide answers the most frequently asked questions and examines alternative options to hiring a formulator (highlighting pros and cons). Includes tips on proactively addressing a few often-overlooked factors that could help make or break both your timeline and budget.

30-page e-book

Three Extracts Guide

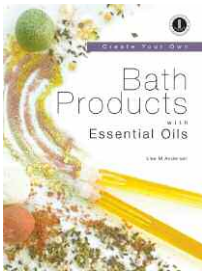
The perfect match to Get Started Making Plant Extracts for Your Cosmetics – try your hand at a few materials and mediums and get a feel for the process while creating bespoke extracts for your artisan cosmetics. Includes step-by-step instruction for 3 extracts, tips, and inspirational photos.

18-page e-book

Make Your Own Preservative Free Natural Cosmetics With 21 Ingredients

This course-in-one-book is a complete beginners guide to making quality skin and haircare products with easy-to-source, inexpensive ingredients. With a total of 21 ingredients and this book, you will be equipped to make a complete collection of personal care products.

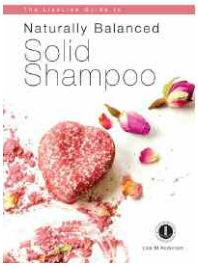
180 page e-book • ALSO AVAILABLE IN SPANISH



Create Your Own Bath Products with Essential Oils

Getting essential oils to disperse safely and completely into a bath can be a challenge. Learn how to make an entire collection of bath products - each one designed to function as an effective base for your own essential oil blends. Easy to make and customizable. No heating involved and no fancy equipment required.

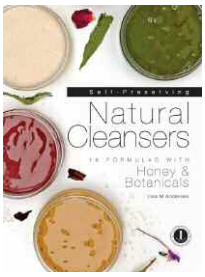
150 page e-book



Make Your Own Naturally Balanced Shampoo Bars

Create your own 'SYNDET' (synthetic detergent) shampoo bars using botanicals and gentle surfactants. A collection of 7 unique formulas with something for every hair and scalp type. All formulas are preservative free and have shelf life of at least 6 months. Every bar is pH balanced to be scalp and hair friendly.

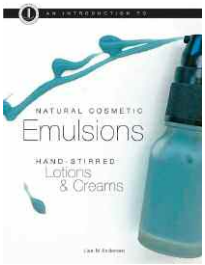
70 page e-book



Self-Preserving Natural Cleansers - 16 Formulas with Honey and Botanicals

Learn the secret of making self-preserving cleansers using pure, natural ingredients and readily available tools. This book shows you how to transform nature's best offerings into a series of 16 unique cleansers that do double duty as mask treatments. Includes a formulation guideline so you can create your own honey-based cleansers and masks.

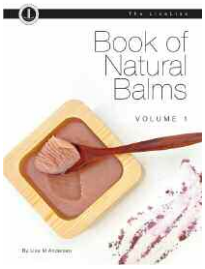
120 page e-book



An Introduction to Natural Cosmetic Emulsions - Hand-Stirred Lotions & Creams

Learn how uncomplicated it can be to make luxurious lotions and stunning creams using your favorite natural ingredients and a few simple tools. This e-book includes a 'toolkit' of preservative choices, 6 different emulsifiers, and loads of tried and true techniques and methods. Richly illustrated. Suitable for most skill levels.

178 page e-book



The LisaLise Book of Natural Balms - Volume 1

Create a series of eight stunning water-free products from anhydrous 'cream' to naturally flavored lip balms using your own handcrafted infusions. Includes a 24 page section on oil infusion methods. All formulas are preservative free and have at least 6 months shelf life.

140 page e-book