Novel preparation for liquid crystal mixture of plant-derived water-soluble, antimicrobial medium-chain monoglycerides

J. Mosbaugh, P. Calvert

LAUREST® — Biopolysan®

Introduction

Medium-chain fatty acid esters, such as glycerol monolaurate (GML) and lauric esters (LE), which can be derived from coconut or palm kernel oil, are well known to possess antimicrobial properties in vitro. GML (also known as monolaurin) has been studied for over three decades and has been shown to be the most active medium-chain ester against bacteria and fungi[1-26]. Commonly used in cosmetics and food products, GML has a known safety record. In 2016, the Personal Care Products Council released a Cosmetic Ingredient Review by expert panel with the first guidance for use.[26]

Antimicrobial Properties of GML

GML is effective against:

- *Staphylococcus aureus (S. aureus)* including methicillin resistant *S. aureus*[2,7,11]
- *Mycobacterium terrae (M. terrae)* [2]
- *Helicobacter pylori (H. pylori)*[2]
- *Staphylococcus epidermidis (S. epidermidis)*[7]
- *Streptococcus mutans (S. mutans)*[15]
- *Candida albicans*[1] *(C. albicans)*
- *Listeria monocytogenes*[17]
- Ribonucleic acid-enveloped viruses[4]
- Deoxyribonucleic acid-enveloped viruses[4]

In a study of medium-chain fatty acids and 1-monoglycerides:

- GML was the most active compound against *Staphylococcus, Corynebacterium, Bacillus, Listeria* and *Streptococcus*
- GML was synergistic with monocaprin (C10) and lauric acid, which was shown to be poorly active on its own.[6]

GML has exhibited excellent antibacterial properties against methicillin-sensitive *S. aureus* (MSSA), methicillin resistant *S. aureus* (MRSA), and *S. epidermidis*. [7]

Uses that have been investigated include:

Superficial skin infections:

- Statistically significant *in vitro* broad-spectrum sensitivity against Gram-positive and Gram-negative bacterial isolates from superficial skin infections.
- Most bacteria did not exhibit resistance to GML.[3]

Controls vaginal flora:

- GML is effective in controlling vaginal flora.
- It is candicidal but did not alter vaginal pH or *lactobacillus* populations.[1]

Prevents infection associated with orthopaedic hardware:

- GML-coated Kirschner wires prevent infection after insertion of orthopaedic external fixators. [7]

Food preservation:

- A study comparing GML, ethanol and lactic acid against *L. monocytogenes*
- GML showed the lowest minimum inhibitory concentration (MIC): 10 µg/mL.[17]

Inhibits biofilm formation.[8, 9, 11, 12, 14]

- GML inhibited biofilm development with two strains of *S. aureus*
- As little as 0.35 mM GML inhibited biofilm development by at least 100,000-fold[11]
- Synergistic use of GML and a peptidoglycan hydrolase control biofilms associated with dental caries: over 72 hours reduced *S. mutans* to levels insufficient to support demineralization of the tooth. [15]

Challenges Utilizing GML's Antimicrobial Properties

The mode of action of the antimicrobial activity of GML is hypothesized to be due to membrane active surfactant properties typical of this class of compounds. The morphological response to these compounds results in membrane destabilization.[10,11,14] The manner in which GML is incorporated into a formulation is found to be a critical aspect in preserving the innate and unique antimicrobial properties.

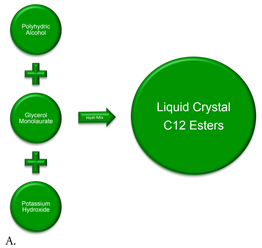
Limited solubility in water, alcohol, and other lipids requires that GML be melted (~ 60 ºC) and then combined with various emulsifiers and/or surfactants to create stable emulsions. These processes result in muted or *completely negated* antimicrobial actions although the emollient nature of GML is maintained.

The type of emulsion factors strongly into GML's efficacy, specifically micelle morphology and size. Lopes et al. showed that GML nanocapsules significantly reduced the biomass of *C. albicans* biofilm while non-nanoencapsulated GML does not exhibit the same effect.[18]

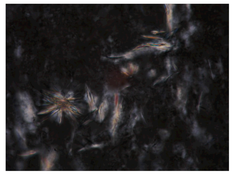
Our Methodology

Copperhead Chemical Company® developed and patented a method that results in a liquid crystal, water-soluble mixture of GML C12 esters that preserves the natural antimicrobial efficacy of GML. The resulting ingredient is useful for cosmetics, personal care, over-the-counter drug, and other therapeutic products.

In general, the method to make the liquid crystal mixture of medium-chain fatty acid esters can be represented by Figure 1.A.

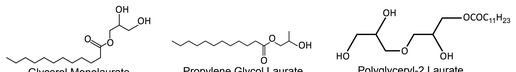


A.



B.

Fig. 1. A. GML, in the presence of a polyhydric alcohol such as propylene glycol or polyglyceryl-2, when heated with the alkali catalyst potassium hydroxide results in the partial saponification and transesterification of GML to the corresponding esters.
B. Cross-polarized microscopy image of Biopolysan® (BPS) @ 9.8 ºC.



More specifically, a liquid crystal mixture of water-soluble polyglyceryl-n esters is described below.

Materials

Glycerol monolaurate, CAS number 142-18-7, was procured from Colonial Chemical Inc. Diglycerin, CAS number 56-81-5 was procured from Spiga Nord and/or Solvay. Potassium hydroxide 45% solution, CAS number 1310-58-3 was procured from Florida Chemical Supply.

Method

- Melt stoichiometrically specific amounts of GML in diglycerin in the presence of potassium hydroxide (45%) with constant low shear mixing.
- Heat mixture to 110 ºC and hold for up to 1 hour.
- The mixture is initially cloudy but clears as the reactions complete.
- Check mixture to specification via Fourier-transform infrared spectroscopy, pH, and successful MIC against *S. aureus.*

The reaction takes place in in a single reactor and all ingredients are conserved. There is no waste stream from the process and there is no solvent, acid or caustic treatment required. Temperatures for processing range from 65 ºC to 115 ºC depending upon the type of polyhydric selected for the reaction. Commercial ingredients currently available are:

Laurest® 1220	INCI: Diglycerin, Polyglyceryl-2 Laurate
Biopolysan® 120	INCI: Propylene Glycol, Propylene Glycol Laurate
Biopolysan® 220B	INCI: Benzyl Alcohol, Diglycerin, Polyglyceryl-2 Laurate

Results

The liquid crystal mixtures, Biopolysan® 120 and Laurest® 1220, underwent *in vitro* testing for antimicrobial efficacy via a third party laboratory at Microchem® Laboratories located in Round Rock, Texas.

Compared to their GML precursor, these liquid crystal mixtures exhibit broader range antimicrobial efficacy in a user-friendly form showing strong antimicrobial action against enveloped microorganisms, such as Gram-positive bacteria, fungi and enveloped viruses.

Fig. 2 Biopolysan® 120 Typical MIC values against common bacteria and fungi

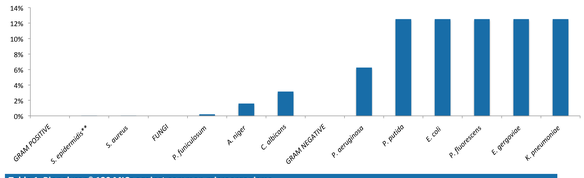


Table 1 Biopolysan® 120 MICs against common microorganisms

Test Sample	Test Microorganism	MIC	Positive Control	Negative Control
Biopolysan® 120	*S. aureus* ATCC 6538	0.05%	Growth	No Growth
	S. epidermidis ATCC 12228	0.05%	Growth	No Growth
	C. albicans ATCC 10231	3.13%	Growth	No Growth
	A. niger ATCC 6275	≥1.60%	Growth	No Growth
	P. funiculosum ATCC 11797	0.20%	Growth	No Growth

Laurest® 1220 MICs returned nearly identical results as Biopolysan, showing that the greatest inhibitory action was against Gram-positive bacteria, followed by fungi, and then relatively high concentrations required to inhibit Gram-negative. Typical Laurest® 1220 inhibitory concentrations required to control Gram negative bacteria ranged from 6.5% to >12%.

Table 2 shows Laurest® 1220's MICs against selected Gram-positive bacteria. Of particular interest is the MIC against *Propionibacterium acnes* (*P. acnes*) of 0.39%, which is in MIC range of common over the counter anti-acne product actives such as benzoyl peroxide (0.13-0.026%)[29, 30] and salicylic acid (0.064%)[31].

Table 2 Laurest® 1220 MICs against Gram positive bacteria

Test Substance	Test Microorganism	MIC	Positive Control	Negative Control
Laurest® 1220	*S. aureus* ATCC 6538	0.049%	Growth	No Growth
	S. epidermidis ATCC 12228	0.049%	Growth	No Growth
	P. acnes ATCC 6919	0.390%	Growth	No Growth

These ingredients are particularly effective against fungi, especially the dermatophytic fungi commonly associated with infection such as *Trichophyton mentagrophytes (T. ment)*, a common dermatophytic fungus associated with toe nail and skin infections. Testing showed a 99.98% reduction in *T. ment* concentration vs. the control after one hour of contact with BPS.

Irritation studies on Laurest® 1220 (MatTek Epi-Derm™ *(in vitro)* and Biopolysan® 120 (Human Repeat Insult Patch Test) showed that they are are non-irritating up to 12% (maximum concentration tested).

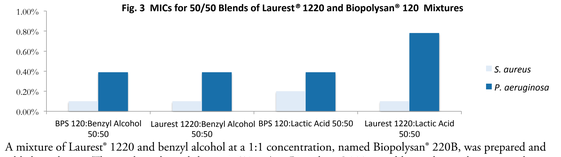
Beneficial Properties

- Formulated with plant-derived esters
- Laurest® 1220 and Biopolysan® 220 are Ecocert Greenlife COSMOS-compliant natural raw materials
- Easy to use
- Water-soluble - cold process capable
- Antimicrobial properties
- Skin conditioning - emollient and humectant
- Non-irritating
- Excellent solvent or carrier for fragrances or active ingredients - other botanicals, essential oils, other actives
- Non-ionic emulsifier and mild surfactant
- Dispersant properties
- Wetting agent
- Stabilizer and pH modifier

Preservation

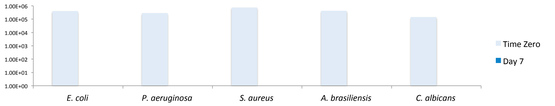
On their own, GML and related esters are primarily effective against Gram-positive bacteria and certain fungi. Ideal for use in therapeutic products such as anti-acne and anti-fungal products.
Mixtures of these esters with common organic alcohol increases efficacy against Gram-negative bacteria.

Fig. 3 MICs for 50/50 Blends of Laurest® 1220 and Biopolysan® 120 Mixtures



A mixture of Laurest® 1220 and benzyl alcohol at a 1:1 concentration, named Biopolysan® 220B, was prepared and added to a lotion. The results indicated that at 1.5% wt/wt, Biopolysan® 220 was able to adequately preserve the product and signals that these chemistries may be part of useful strategies to naturally preserve personal care products.

Fig. 4 Preservative Screen Study ; Sample L-220B-A (1.5 wt/wt% Biopolysan® 220B)



Benefits to the Microbiome

The novel and selective antimicrobial actions of these liquid crystal forms of GML and related esters present an opportunity for a significant impact on diversity of the skin microbiome which can translate to therapeutic benefits beyond the scope of moisturizing and skin conditioning.

Lauric acid is one of the Free Fatty Acids (FFA) in human sebum. FFA are secreted in triglyceride form, which commensal bacteria hydrolyze, releasing monoglycerides and other FFAs. Lauric acid has direct antimicrobial action in and on the skin and is the strongest of the skins natural antimicrobial substances in sebum [27,28]

These ingredients are being studied to further explore their impact on the natural diversity of the skin microbiome. In addition to promoting optimal skin health and beauty, Laurest® 1220 and Biopolysan® 120 may have a role in addressing atopic dermatitis, eczema, acne vulgaris, fungal infections, and other skin conditions.

COPPERHEAD CHEMICAL COMPANY®

References:
1. Strandberg KL, Peterson ML, Lin YC, Pack MC, Chase DJ, Schlievert PM. Glycerol monolaurate inhibits Candida and Gardnerella vaginalis in vitro and in vivo but not Lactobacillus. Antimicrob Agents Chemother. 2010;54(2):597-601.Epub 2009 Dec 14.
2. Preuss HG, Echard B, Enig M, Brook I, Elliott TB. Minimum inhibitory concentrations of herbal essential oils and monolaurin for gram-positive and gram-negative bacteria. Mol Cell Biochem. 2005;272(1-2):29-34.DOI: 10.1007/s11010-005-6604-1
3. Carpo BG, Verallo-rowell VM, Kabara J. Novel antibacterial activity of monolaurin compared with conventional antibiotics against organisms from skin infections: an in vitro study. J Drugs Dermatol. 2007;6(10):991-8.
4. Hierholzer JC, Kabara JJ. In Vitro effects of monolaurin compounds on enveloped RNA and DNA viruses. J Food Saf. 1982;4:1-12.
5. Strandberg KL, Peterson ML, Schaefers MM, et al. Reduction in Staphylococcus aureus growth and exotoxin production and in vaginal interleukin 8 levels due to glycerol monolaurate in tampons. Clin Infect Dis. 2009;49(11):1711-7.
6. Batovska DI, Todorova IT, Tsvetkova IV, Najdenski HM. Antibacterial study of the medium chain fatty acids and their 1-monoglycerides: individual effects and synergistic relationships. Pol J Microbiol. 2009;58(1):43-7.
7. Gil D, Shuvaev S, Frank-kamenetskii A, Reukov V, Gross C, Vertegel A. Novel Antibacterial Coating on Orthopedic Wires To Eliminate Pin Tract Infections. Antimicrob Agents Chemother. 2017;61(7).
8. Ham Y, Kim TJ. Inhibitory activity of monoacylglycerols on biofilm formation in Aeromonas hydrophila, Streptococcus mutans, Xanthomonas oryzae, and Yersinia enterocolitica. Springerplus. 2016;5(1):1526.
9. Sekeen D, Chen E, Benso B, Pardi V, Murata RM. In vitro evaluation of antifungal activity of monolaurin against Candida albicans biofilms. PeerJ. 2016;4:e2148.
10. Yoon BK, Jackman JA, Kim MC, Cho NJ. Spectrum of Membrane Morphological Responses to Antibacterial Fatty Acids and Related Surfactants. Langmuir. 2015;31(37):10223-32.
11. Hess DJ, Henry-stanley MJ, Wells CL. The Natural Surfactant Glycerol Monolaurate Significantly Reduces Development of Staphylococcus aureus and Enterococcus faecalis Biofilms. Surg Infect (Larchmt). 2015;16(5):538-42.
12. Mueller EA, Schlievert PM. Non-aqueous glycerol monolaurate gel exhibits antibacterial and anti-biofilm activity against Gram-positive and Gram-negative pathogens. PLoS ONE. 2015;10(3):e0120280.
13. Tangwatcharin P, Khopaibool P. Activity of virgin coconut oil, lauric acid or monolaurin in combination with lactic acid against Staphylococcus aureus. Southeast Asian J Trop Med Public Health. 2012;43(4):969-85.
14. Schlievert PM, Peterson ML. Glycerol monolaurate antibacterial activity in broth and biofilm cultures. PLoS ONE. 2012;7(7):e40350.
15. Lester K, Simmonds RS, Zoocin A and lauricidin in combination reduce Streptococcus mutans growth in a multispecies biofilm. Caries Res. 2012;46(3):185-93.
16. Calvert P, Cappuccino CD, Mosbaugh J, Phelan EL, inventors; Copperhead Chemical Co Inc, assignee. Methods and compositions for novel liquid crystal delivery systems. US Patent 8,546,593. October 1, 2013.
17. Oh DH, Marshall DL. Antimicrobial activity of ethanol, glycerol monolaurate or lactic acid against Listeria monocytogenes. Int J Food Microbiol. 1993;20(4):239-46.
18. Lopes LQ, Santos CG, Vaucher Rde A, Raffin RP, Santos RC. Nanocapsules with glycerol monolaurate: Effects on Candida albicans biofilms. Microb Pathog. 2016;97:119-24. Epub 2016 May 27.
19. Fessi H, Puisieux F, Devissaguet J.Ph, Ammoury N, Benita S. Nanocapsule formation by interfacial polymer deposition following solvent displacement. Int. J. Pharm. 1989; 55(1):R1-4.
20. Projan SJ, Brown-skrobot S, Schlievert PM, Vandenesch F, Novick RP. Glycerol monolaurate inhibits the production of beta-lactamase, toxic shock toxin-1, and other staphylococcal exoproteins by interfering with signal transduction. J Bacteriol. 1994;176(14):4204-9.
21. Ruzin A, Novick RP. Glycerol monolaurate inhibits induction of vancomycin resistance in Enterococcus faecalis. J Bacteriol. 1998;180(11):182-5.
22. Schlievert PM, Deringer JR, Kim MH, Projan SJ, Novick RP. Effect of glycerol monolaurate on bacterial growth and toxin production. Antimicrob Agents Chemother. 1992;36(3):626-31.
23. Schlievert PM, Blomster DA. Production of staphylococcal pyrogenic exotoxin type C: influence of physical and chemical factors. J Infect Dis. 1983;147(2):236-42.
24. Final report of the amended safety assessment of Glyceryl Laurate. Int J Toxicol. 2004;23 Suppl 2:55-94.
25. Boughton B, Wheatley VR. The Fatty Acid Composition of Skin Surface Fat ("Sebum") of Normal Human Subjects. J Invest Dermatol. 1959; 33(2): 49-55.
26. Yang D, Pornpattananangkul D, Nakatsuji T, et al. The antimicrobial activity of liposomal lauric acids against Propionibacterium acnes. Biomaterials. 2009;30(30):6035-40.
27. Okamoto K, Ikeda F, Kanzyama S, et al. In vitro antimicrobial activity of benzoyl peroxide against Propionibacterium acnes assessed by a novel susceptibility testing method. J Infect Chemother. 2016;22(6):426-9.. doi: 10.1016/j.jiac.2015.12.010.
28. Pannu J, Mccarthy A, Martin A, et al. In vitro antibacterial activity of NB-003 against Propionibacterium acnes. Antimicrob Agents Chemother. 2011;55(9):4211-7. doi: 10.1128/AAC.00561-11.
29. Desbois AP, Lawlor KC. Antibacterial activity of long-chain polyunsaturated fatty acids against Propionibacterium acnes and Staphylococcus aureus. Mar Drugs. 2013;11(11):4544-57.
30. United States Pharmacopeia and National Formulary. (USP29-NF24) Page 2499. http://www.pharmacopeia.cn/v29240/usp29nf24s0_c51.html