Surfactant Concentration and Type Affects the Removal of Escherichia coli from Pig Skin During a Simulated Handwash

Running Head: Surfactants and bacteria removal

Surfactant Concentration and Type Affects the Removal of *Escherichia coli* from Pig Skin During a Simulated Handwash

Dane A. Jensen*, Michael A. Rogers, and Donald W. Schaffner

Food Science Department, Rutgers University, 65 Dudley Rd., New Brunswick, NJ, 08901-8520.

***Author for correspondence:** Tel: +1 (732) 649-2573; Email: dane.jensen@rutgers.edu

Significance and Impact of Study

This study characterizes the role of surfactants in removing microbes during a handwash. Numerous studies address how surfactants support antimicrobial effect in soap, or cause irritation of skin, but no published studies show which surfactants are best for removing microbes. We used pig skin as a model for human skin and a lathering device to simulate a hand wash. A 10% sodium lauryl sulfate mixture was the only treatment significantly different from a water wash. There was a strong correlation between increasing surfactant concentrations above the critical micelle concentration and mean microbial reduction.

Abstract

The effect of surfactant type and concentration on a bland soap formulations ability to remove bacteria from hands remains largely unstudied. Several combinations of surfactants and water were combined to test bacterial removal efficacy using a handwashing device (two pieces of pig skin and a mechanical motor) to simulate a handwash. A nalidixic acid resistant, non-pathogenic strain of *Escherichia coli* (ATCC 11229) was used. Two anionic surfactants, sodium lauryl sulfate and sodium stearoyl lactylate, and two nonionic surfactants, poloxamer 407 and sorbitan monostearate, each in concentrations of 2%, 5%, and 10% were studied. A slight positive (r^2=0.17) but significant (p=0.03) correlation was observed between hydrophile-lipophile balance value and mean log reduction. No correlation was observed between pH of the treatment solution and the mean log reduction (r^2=0.05, p=0.25). A 10% sodium lauryl sulfate mixture showed the highest log reduction ($\bar{x}$= 1.1 log cfu reduction, SD=0.54), and was the only treatment significantly different from washing with water (p=0.0005). There was a correlation between increasing surfactant concentrations above the critical micelle concentration, and mean microbial reduction (r^2=0.62, p=0.001).

Key Words: E.coli, Food safety, Environmental health, Microbial contamination, Modelling

Introduction

Proper hand hygiene is important in prevent pathogen transmission (Montville et al. 2002; Green et al. 2007; Strohbehn et al. 2008; Burton et al. 2011). Washing hands with soap generally produces a better microbial reduction than washing hands with just water (Burton et al. 2011), especially if debris is present on hands (Jensen et al. 2015). Many studies have addressed antimicrobial soap formulation, and how antimicrobial soaps may provide a greater bacterial reduction than plain or bland soap (Fischler et al. 2007; Fuls et al. 2008; Montville and Schaffner 2011). Very few studies have examined what surfactants types and concentrations are key for optimal effectiveness in removing microbes from hands, with a limited number of studies examining links between antimicrobial effectiveness and surfactant concentration (Benson et al. 1990; Taylor et al. 2004).

Soap is an alkali salt of a fatty acid (carboxylic acids) that has a hydrophobic hydrocarbon tail and a hydrophilic carboxylate head group (Stache 1995; Lin et al. 2005). These fatty acids can be saturated or unsaturated, and derived from natural or synthetic sources. Fatty acid salts are added to water to create the final soap product. Additional components, such as fillers, dyes, or scents, are often added (Balzer et al. 1995). Surfactants in hand soaps facilitate the formation of micelles which can surround oily debris to allow the debris to be more readily solubilized in water and be removed during a wash (Kawai and Imokawa 1984; Froebe et al. 1990; Piérard et al. 1995; Simion et al. 1991; Aramaki et al. 2001; Gloor et al. 2004; Purohit et al. 2014).

The choice of which surfactant to use in soap is not always directly connected to microbial removal, as skin health, tactile, and formulation stability aspects play a role (Kawai and Imokawa 1984; Froebe et al. 1990; Simion et al. 1991; Aramaki et al. 2001; Gloor et al. 2004; Krawczyk 2015). Penetration of the surfactant into the skin is dependent on exposure time, temperature, and surfactant head group (Mao et al. 2012). Repeated use of soap containing certain surfactants will damage the stratum corneum, which manifests as dry, cracked, and red skin (Kawai and Imokawa 1984; Froebe et al. 1990; Simion et al. 1991; Piérard et al. 1995; Aramaki et al. 2001; Gloor et al. 2004; Krawczyk 2015). Anionic surfactants cause significant damage to skin, with the sodium lauryl sulfate reportedly causing the most irritation (Kawai and Imokawa 1984; Piérard . 1995; Aramaki et al. 2001; Gloor et al. 2004; de Jongh et al. 2006). Sodium lauryl sulfate is an irritant at critical micelle concentrations at or greater than 2% (Robinson et al. 2010). Anionic surfactants remove more of the stratum corneum than cationic surfactants, which are considered gentler on the skin (Ginn et al. 1970; Kawai and Imokawa 1984; Kawasaki et al. 1997; Krawczyk 2015). An argument could be made that surfactants which remove more layers from the stratum corneum would provide a greater microbial reduction during a hand wash (Lowbury et al. 1964; Jumaa 2005).

Results and Discussion

Figure 1 shows the mean log reductions of a water treatment compared to the treatments with different surfactants at each different concentration (2%, 5%, and 10%). The control treatment with water showed a 0.6 ± 0.4 mean log cfu reduction.

A 10% sodium lauryl sulfate solution showed the highest log reduction (1.1 ± 0.5 log cfu), and was the only treatment that was significantly different from water ($p=0.0005$).

The idea that a greater concentration of bacteria on the hand could be removed with increasing concentration of surfactants that remove more of the stratum corneum (Kawai and Imokawa 1984; Froebe et al. 1990; Simion et al. 1991; Piérard et al. 1995; Aramaki et al. 2001; Gloor et al. 2004) is supported by the 10% sodium lauryl sulfate solution data (Figure 1), and by data shown in Figure 2. It was surprising to see the opposite trend for sodium stearoyl lactylate (Figure 1). Studies that characterized the removal of oily debris and skin from hands suggest that anionic surfactants would remove microbes slightly better than nonionic surfactants (Ginn et al. 1970; Krawczyk 2015). Sodium lauryl sulfate outperformed the nonionic surfactants Span 60 and poloxamer 407 here, but sodium stearoyl lactylate did not similarly outperform nonionic surfactants. The hydrophilic-lipophilic balance (HLB) value of sodium stearoyl lactylate offers one explanation. The sodium stearoyl lactylate surfactant would be water-in-oil emulsifier at an HLB value of 10 (Table 1), and the action of lathering the sodium stearoyl lactylate solution could create a barrier on the skin that would prevent the superficial layers of the pig skin from being washed away.

A summary of HLB, pH, and CMC (critical micelle concentration) values for the surfactants studied is in Table 1. A minor ($r^2=0.17$), yet significant ($p=0.03$) positive correlation was seen between HLB value and mean log reduction. No correlations

were seen between pH of the treatment solution and mean log reduction (r^2=0.05, p=0.25). Figure 2 shows a scatter plot of free grams of surfactant in solution, and the observed microbial reduction and shows a positive correlation (r^2=0.62, p=0.001). Free grams of surfactant in solution refer to the amount of surfactants available in 1 mL of surfactant solution that are able to form micelles. The higher the value of free surfactants, the greater microbial reduction observed. Once the critical micelle concentration and free grams in the surfactant solutions are considered, it is clear surfactant concentration plays an important role. Froebe et al. observed concentration dependent lipid removal above the critical micelle concentration (Froebe et al. 1990). Krawczyk determined that a surfactant concentration dependent interaction between the liquid molecules and the skin surface occurred, once the critical micelle concentration was reached (Krawczyk 2015). We hypothesize that increasing the concentration of surfactant has benefit above the critical micelle concentration (listed in Table 1) as observed in Figure 2.

The finding that many surfactant solutions did not show a significant difference from water alone is not surprising. Prior research showed no statistically significant difference between a water or a water and soap wash when there was no oily/fatty debris was present on the subjects' hands (Jensen et al. 2015). The maximum log reduction observed on the treated pig skin was when a surfactant was used was higher than the maximum log reduction observed with a water treatment in the current study. A similar result was observed in Jensen et al. (2015) where the maximum log reduction with soap was higher (~4 log CFU) than when no soap was used (~2 log reduction).

While significant differences between plain water or surfactant solutions were not seen for most of the treatments, these findings, and those from Jensen et al. (2015), would suggest that further research using inoculated pig skin with oil or grease carrier matrix could better reveal the full efficacy of a surfactant solution. A high concentration of sodium lauryl sulfate may promote microbial reduction, due to the repulsive interaction of the anionic molecules with skin (Ginn et al. 1970; Krawczyk 2015). Care must be taken since sodium lauryl sulfate based soap can damage skin at higher concentrations (Kawai and Imokawa 1984; Piérard et al. 1995; Aramaki et al. 2001; Gloor et al. 2004; de Jongh et al. 2006). The concentration of primary surfactant in soap should be higher than the critical micelle concentration, as surfactant concentrations above the critical micelle concentration is strongly correlated with mean microbial reduction.

Methods and Materials

Pig skin was used here to study handwashing efficacy. Several studies have used pig skin as a suitable substrate to test topical surfactant and antimicrobial efficacy (Bush et al. 1986; Benson et al. 1990; Geraldo et al. 2008). The pig skin stratum corneum is >90% cermaide, cholesterol, and fatty acids, and shows no changes with each layer of strateum corneum removed, much like human skin (Gray and Yardley 1975). Details on preparation of the pig skin test substrate are available elsewhere (Bush et al. 1986; Benson et al. 1990). Briefly, the de-haired loin and rib sections of pig skin from several pigs were obtained from a local butcher. Skins were washed with tap water, sized, placed inside plastic bags, and frozen. Antimicrobial activity of

156 pig skin was tested (ASTM International 2008) to ensure any antibiotics present
157 would not affect microbial recovery or survival. Tryptic soy broth (Remel, Thermo
158 Fisher Scientific, Waltham, MA) without pig skin, and tryptic soy broth containing
159 10% homogenized pig skin were inoculated with the test organism (see below) and
160 incubated at 35 °C for 24 h. No difference in growth rate between the samples was
161 observed.

162 *Preparation of pig skin substrate samples:* Frozen pig skin was thawed, and defatted
163 with a sterilized knife. Pig skin was cut into 3x3 cm and 8x3 cm sections. Skin pH
164 was measured using an Accument flat surface pH probe (Fisher Scientific, Waltham,
165 MA), and had a mean pH of 6.9, while human skin has a pH of 4-6 (Rieger 1989;
166 Ehlers et al. 2001).

167 *Bacterial Strain: Escherichia coli* has been shown to be able to survive for several
168 hours on pig skin (Bush et al. 1986). A nalidixic acid resistant mutant of the non-
169 pathogenic strain of *Escherichia coli* (ATCC 11229) commonly used in handwashing
170 experiments (ASTM International 2006) was prepared. The organism was made
171 resistant to 50 µg nalidixic acid by stepwise exposure (Parnell et al. 2005). Nalidixic
172 acid resistance facilitates recovery of the *E. coli* amidst the natural pig skin
173 microbiota. Culturing of *E. coli* was as indicated in ASTM protocols (ASTM
174 International 2006). *E. coli* was cultured in 5 mL soybean-casein digest broth for
175 24±4 h at 35±2 °C. The 24 h culture was harvested by centrifugation (Micro 12,
176 Fisher Scientific) at 7,000 x *g* for 10 min, and washed in phosphate buffer saline
177 (PBS; 0.1M, pH 7.2). Centrifugation and washing was repeated three times. Cell

178 pellets were re-suspended in phosphate buffer saline to form a solution of ~8 log
179 $CFUmL^{-1}$ used to inoculate the pig skin.
180
181 *Surfactants:* Surfactant used in this study are commonly used in personal hygiene
182 products (Jensen, 2015). Two anionic surfactants, sodium lauryl sulfate (Sigma-
183 Aldrich, St. Louis, MO) and sodium stearoyl lactylate (Spectrum Chemical MFG Corp,
184 New Brunswick, NJ); and two nonionic surfactants, poloxamer 407 (Sigma-Aldrich,
185 St. Louis, MO), and sorbitan monostearate (Span 60) (Sigma-Aldrich, St. Louis, MO)
186 were used at concentrations of 2%, 5%, and 10%. The solutions were prepared by
187 combining appropriate amounts of the surfactant and distilled water. Water was
188 boiled in a covered glass beaker for 5 min to inactivate vegetative bacteria, and
189 allowed to cool to ~25 °C. Surfactants were added to the water, covered, then mixed
190 for 5 min. Sorbitan monostearate was not miscible in room temperature water, and
191 was dispersed by warming the water to ~70 °C while mixing (Wu et al. 2010). The
192 solutions of sorbitan monostearate solutions stayed dispersed for the duration of
193 the experiment (~ 1 h). The critical micelle concentration of surfactants in water (%
194 solution) was determined via the pendant drop method using a goniometer (Ramé-
195 hart, Succasunna, NJ) and surface tension software (KSV Surface Tension Software,
196 Biolin Scientific, Stockholm, Sweden) at room temperature, ~25 °C (Mukerjee and
197 Mysels 1971), and the calculated critical micelle concentration values were verified
198 using the published literature.

199 *Lathering device:* A mechanical lathering device was fabricated to simulate
200 handwashing under controlled pressure and shear stress. The device consisted of

two horizontal stainless steel metal plates, where the bottom plate (2.5x3 cm) is moved back and forth (18 RPM) by a simple rotational motor, while the top plate (5x3 cm) remained fixed. The two plates were pressed together using a 500 g weight.

Inoculation of test substrate: The bottom pig skin piece was inoculated with 1mL of ~7 log CFU mL^{-1} solution of *E. coli*, and rubbed against the top piece for 30 s to distribute the inoculum. The pig skin was allowed to dry for ~60 s, until no moisture was visible.

Prewash sampling: Top and bottom pieces of pig skin were placed in Whirl-Pak filter bags (Nasco, Fort Atkinson, WI, US) with phosphate buffer saline. The substrate and buffer were homogenized (Stomacher, Dynatech Laboratories, Alexandria, VA) for 2 min. The solution was diluted in a phosphate saline buffer, and plated onto BBL™ MacConkey agar containing 30 μg nalidixic acid mL^{-1} media.

Washing: Two sections of inoculated pig skin were attached to the device. One mL of tap water (control) or surfactant solution were dispensed onto the pig skin and the device oscillated for 10 s at 18 RPM. Pig skin was removed from the device using sterile forceps and rinsed for 10 s with plain tap water at ~26 °C. Rinsed pig skins were placed in a 207 mL Whirl-Pak filter bag (Nasco, Fort Atkinson, WI, US), with phosphate buffer saline and homogenized for 2 min. The solution was diluted in phosphate buffer saline (pH 7.2 ±0.1), plated on BBL™ MacConkey agar containing 30 μgmL^{-1} nalidixic acid, and incubated at 35°C for 24 h.

Pre and post wash differences were calculated, and frequency histograms were constructed using Excel (Microsoft, Redmond Washington). ANOVA and Tukey's honest significance difference test (MATLAB, Natick, Massachusetts) were used to determine if multiple means were significantly different and if any significant interactions existed between the variables.

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Conflicts of Interest

No conflict of interest exist.

References

Aramaki, J., Löffler, C., Kawana, S., Effendy, I., Happle, R. and Löffler, H. (2001) Irritant patch testing with sodium lauryl sulphate: interrelation between concentration and exposure time, *Br J Dermatol*, 145(5), pp. 704-8.

ASTM International (2006) E1174 – 13 Standard Test Method for Evaluation of the Effectiveness of Health Care Personnel Handwash Formulations, ASTM International, West Conshohocken, PA.

ASTM International (2008) E 1054 – 08. Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents, ASTM International, West Conshohocken, PA.

Ayer, J. and Maibach, H.I. (2008) Human skin buffering capacity against a reference base sodium hydroxide: in vitro model, *Cutan Ocul Toxicol*, 27(4), pp. 271-81.

Balzer, D., Varwig, S. and Weihrauch, M. (1995) Viscoelasticity of personal care products, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 99(2), pp. 233-46.

Benson, L., LeBlanc, D., Bush, L. and White, J. (1990) The effects of surfactant systems and moisturizing products on the residual activity of a chlorhexidine gluconate handwash using a pigskin substrate, *Infect Control Hosp Epidemiol*, 11(2), pp. 67-70.

Burton, M., Cobb, E., Donachie, P., Judah, G., Curtis, V. and Schmidt, W.P. (2011) The effect of handwashing with water or soap on bacterial contamination of hands, *Int J Environ Res Public Health*, 8(1), pp. 97-104.

Bush, L.W., Benson, L.M. and White, J.H. (1986) Pig skin as test substrate for evaluating topical antimicrobial activity, *J Clin Microbiol*, 24(3), pp. 343-8.

Ehlers, C., Ivens, U.I., Møller, M.L., Senderovitz, T. and Serup, J. (2001) Females have lower skin surface pH than men, *Skin Res Technol*, 7(2), pp. 90-4.

Fischler, G.E., Fuls, J.L., Dail, E.W., Duran, M.H., Rodgers, N.D. and Waggoner, A.L. (2007) Effect of hand wash agents on controlling the transmission of pathogenic bacteria from hands to food, *J Food Prot*, 70(12), pp. 2873-7.

Froebe, C.L., Simion, F.A., Rhein, L.D., Cagan, R.H. and Kligman, A. (1990) Stratum corneum lipid removal by surfactants: relation to in vivo irritation, *Dermatologica*, 181(4), pp. 277-83.

Fuls, J.L., Rodgers, N.D., Fischler, G.E., Howard, J.M., Patel, M., Weidner, P.L. and Duran, M.H. (2008) Alternative hand contamination technique to compare the activities of antimicrobial and nonantimicrobial soaps under different test conditions, *Appl Environ Microbiol*, 74(12), pp. 3739-44.

267 Geraldo, I.M., Gilman, A., Shintre, M.S. and Modak, S.M. (2008) Rapid antibacterial
268 activity of 2 novel hand soaps: evaluation of the risk of development of bacterial
269 resistance to the antibacterial agents, *Infect Control Hosp Epidemiol*, 29(8), pp. 736-
270 41.

271 Ginn, M.E., Dunn, S.C. and Jungermann, E. (1970) Contact angle studies on viable
272 human skin. II. Effect of surfactant ionic type in pretreatment, *J Am Oil Chem Soc*,
273 47(3), pp. 83-5.

274 Gloor, M., Senger, B., Langenauer, M. and Fluhr, J.W. (2004) On the course of the
275 irritant reaction after irritation with sodium lauryl sulphate, *Skin Res Technol*, 10(3),
276 pp. 144-8.

277 Gray, G.M. and Yardley, H.J. (1975) Lipid compositions of cells isolated from pig,
278 human, and rat epidermis, *J Lipid Res*, 16(6), pp. 434-40.

279 Green, L.R., Radke, V., Mason, R., Bushnell, L., Reimann, D.W., Mack, J.C., Motsinger,
280 M.D., Stigger, T. and Selman, C.A. (2007) Factors related to food worker hand
281 hygiene practices, *J Food Prot*, 70(3), pp. 661-6.

282 Jensen, Dane A. Danyluk, Michelle D., Harris, Linda J., and Schaffner, Donald W.
283 (2015) Quantifying the Effect of Hand Wash Duration, Soap Use, Ground Beef Debris,
284 and Drying Methods on the Removal of *Enterobacter aerogenes* on Hands. *J Food*
285 *Prot* 78(4): pp. 685-690.

286 Jensen D.A. (2015) Addressing challenges and gaps in the hand hygiene literature
287 using novel quantitative approaches, Rutgers, The State University of New Jersey,
288 *Graduate School - New Brunswick Electronic Theses and Dissertations*.

289 de Jongh, C.M., Jakasa, I., Verberk, M.M. and Kezic, S. (2006) Variation in barrier
290 impairment and inflammation of human skin as determined by sodium lauryl
291 sulphate penetration rate, *Br J Dermatol*, 154(4), pp. 651-7.

292 Jumaa, P.A. (2005) Hand hygiene: simple and complex, *Int. J. Infect. Dis*, 9(1), pp. 3-
293 14.

294 Kawai, M. and Imokawa, G. (1984) The induction of skin tightness by surfactants, *J.*
295 *Soc. Cosmet. Chem*, 35, pp. 147-56.

296 Kawasaki, Y., Quan, D., Sakamoto, K. and Maibach, H.I. (1997) Electron resonance
297 studies on the influence of anionic surfactants on human skin, *Dermatology*, 194(3),
298 pp. 238-42.

299 Krawczyk, J. (2015) Surface free energy of the human skin and its critical surface
300 tension of wetting in the skin/surfactant aqueous solution/air system, *Skin Res*
301 *Technol*: 21:214-223.

302 Lin, B., McCormick, A.V., Davis, H.T. and Strey, R. (2005) Solubility of sodium soaps
303 in aqueous salt solutions, *J Colloid Interface Sci.*, 291(2), pp. 543-9.

304 Lowbury, E.J.L., Lilly, H.A. and Bull, J.P. (1964) Disinfection of hands: removal of
305 transient organisms, *Br Medical J*, 2, pp. 230-3.

306 Mao, G., Flach, C.R., Mendelsohn, R. and Walters, R.M. (2012) Imaging the
307 distribution of sodium dodecyl sulfate in skin by confocal Raman and infrared
308 microspectroscopy, *Pharm Res.*, 29(8), pp. 2189-201.

309 Montville, R. and Schaffner, D.W. (2011) A meta-analysis of the published literature
310 on the effectiveness of antimicrobial soaps, *J Food Prot*, 74(11), pp. 1875-82.

311 Montville, R., Chen, Y. and Schaffner, D.W. (2002) Risk assessment of hand washing
312 efficacy using literature and experimental data, *Int J Food Microbiol*, 73(2-3), pp.
313 305-13.

314 Mukerjee, A. and Mysels, K. (1971) Critical Micelle Concentrations of Aqueous
315 Surfactant Systems, *National Bureau of Standards, U.S. Government Printing Office,*
316 *Washington, DC*.

317 Parnell, T.L., Harris, L.J. and Suslow, T.V. (2005) Reducing *Salmonella* on cantaloupes
318 and honeydew melons using wash practices applicable to postharvest handling,
319 foodservice, and consumer preparation, *Int J Food Microbiol*, 99(1), pp. 59-70.

320 Piérard, G.E., Goffin, V., Hermanns-Lê, T., Arrese, J.E. and Piérard-Franchimont, C.
321 (1995) Surfactant-induced dermatitis: comparison of corneosurfametry with
322 predictive testing on human and reconstructed skin, *J Am Acad Dermatol*, 33(3), pp.
323 462-9.

324 Purohit, P., Chandar, P., Vilinska, A., Ananthapadmanabhan, K.P. and Somasundaran,
325 P. (2014) Effect of mixed surfactants on stratum corneum: a drying stress and
326 Raman spectroscopy study, *Int J of Cosmetic Sci*, 36(4), pp. 379-85.

327 Rieger, M. (1989) The apparent pH on the skin: Careful quantitative chemical
328 measurements are needed to draw conclusions of this acid/base phenomenon,
329 *Cosmetics and Toiletries*, 104(3), pp. 53-60.

330 Robinson, Valerie C., Wilma F. Bergfeld, Donald V. Belsito, Ronald A. Hill, Curtis D.
331 Klaassen, James G. Marks, Ronald C. Shank, Thomas J. Slaga, Paul W. Snyder, and F.
332 Alan Andersen. (2010) Final report of the amended safety assessment of sodium
333 laureth sulfate and related salts of sulfated ethoxylated alcohols. *Int J Toxicol* 29 (4),
334 pp. 151-161.

335 Simion, F.A., Rhein, L.D., Grove, G.L., Wojtkowski, J.M., Cagan, R.H. and Scala, D.D.
336 (1991) Sequential order of skin responses to surfactants during a soap chamber
337 test, *Contact Dermatitis*, 25(4), pp. 242-9.

338 Stache, H.W. (1995) *Anionic surfactants: organic chemistry,* CRC Press,.

339 Strohbehn, C., Sneed, J., Paez, P. and Meyer, J. (2008) Hand washing frequencies and
340 procedures used in retail food services, *J Food Prot*, 71(8), pp. 1641-50.

341 Taylor, T.J., Seitz, E.P., Fox, P., Fischler, G.E., Fuls, J.L. and Weidner, P.L. (2004)
342 Physicochemical factors affecting the rapid bactericidal efficacy of the phenolic
343 antibacterial triclosan, *Int J Cosmetic Sci*, 26(3), pp. 111-6.

344 Wu, Y., Iglauer, S., Shuler, P., Tang, Y. and Goddard III, W.A. (2010) Alkyl
345 Polyglycoside-Sorbitan Ester Formulations for Improved Oil Recovery, *Tenside*
346 *Surfactants Detergents*, 47(5), pp. 280-7.

347 Zhai, H., Chan, H.P., Farahmand, S. and Maibach, H.I. (2009) Measuring human skin
348 buffering capacity: an in vitro model, *Skin Res Technol*, 15(4), pp. 470-5.

349 Zheng, Y., Sotoodian, B., Lai, W. and Maibach, H.I. (2012) Buffering capacity of
350 human skin layers: in vitro, *Skin Res Technol*, 18(1), pp. 114-9.

351

Figure Legends

Figure 1. Mean log reduction due to treatments with Water, Sodium lauryl sulfate (SLS), Sodium stearoyl lactylate (SSL), Sorbitan monostearate (Sp60), and Poloxamer 407 (p407). The concentrations are in parenthesis. Brackets represent the standard error of the mean.

Figure 2. Correlation between free grams of surfactant and mean log reduction. Surfactants below the critical micelle reported as zero free grams of surfactant. Open shapes are 2% solutions, solid gray are 5% solutions, and solid black shapes are 10% solutions. Sodium lauryl sulfate (△), Sodium stearoyl lactylate (▽), Poloxamer 407 (◇), Sorbitan monostearate (□). The Sodium stearoyl lactylate (5%) and the Sorbitan monostearate (2%) had similar values, so the Sodium stearoyl lactylate (5%) data point is slightly obscured.

Table 1. Characteristics of surfactants and surfaces used in this study. Hydrophilic-lipophilic balance (HLB) and Critical Micelle Concentration (CMC) values are for surfactant solutions only. Human skin was not used in this study, but is shown for reference.

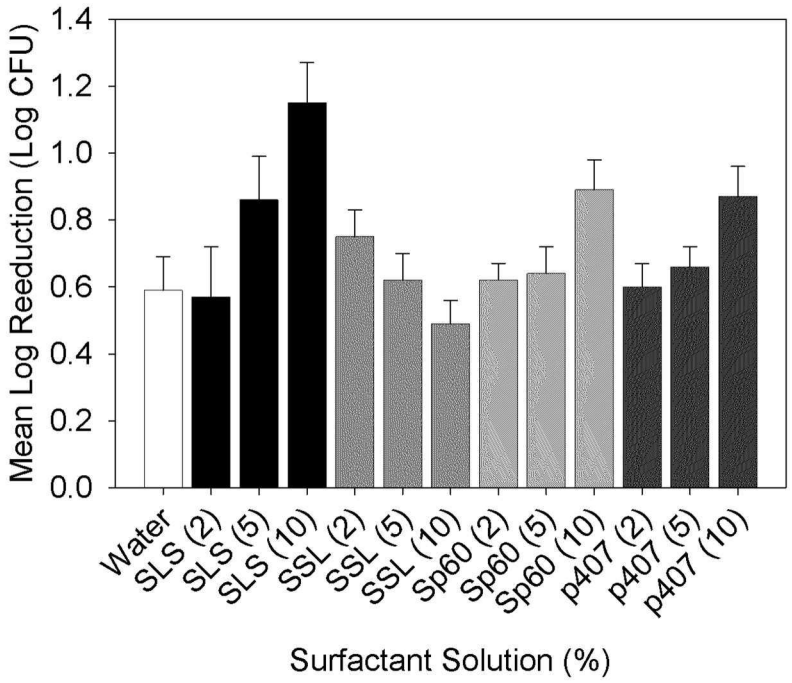
Surfactant or Surface	pH	HLB	CMC (% solution)	Free grams in 1mL solution
Sodium lauryl sulfate 2%	9.2	40.0	0.2	0.018
Sodium lauryl sulfate 5%	9.7	40.0	0.2	0.048
Sodium lauryl sulfate 10%	9.9	40.0	0.2	0.098
Sodium stearoyl lactylate 2%	4.5	10.0	7.0	0.000
Sodium stearoyl lactylate 5%	4.5	10.0	7.0	0.000
Sodium stearoyl lactylate 10%	4.3	10.0	7.0	0.030
Polaxamer 407 2%	6.7	23.0	0.5	0.015
Polaxamer 407 5%	6.9	23.0	0.5	0.045
Polaxamer 407 10%	6.9	23.0	0.5	0.095
Sorbitan monostearate 2%	6.6	4.7	2.0	0.000
Sorbitan monostearate 5%	6.3	4.7	2.0	0.030
Sorbitan monostearate 10%	5.9	4.7	2.0	0.080
Water (control)	6.7	-	-	-
Pig skin	6.9	-	-	-
Human skin*	4-6	-	-	-
Cadaver Skin†	5.9	-	-	-

*Data obtained from (Ehlers et al. 2001; Rieger 1989)

† Data obtained from (Ayer and Maibach 2008; Zhai et al. 2009; Zheng et al. 2012).

Figure 1. Comparison of the mean log reduction from treatments with different surfactant type and concentration. The black brackets represent the standard error of the mean. Each surfactant is presented in order of increasing surfactant concentration (%).

Fig 1

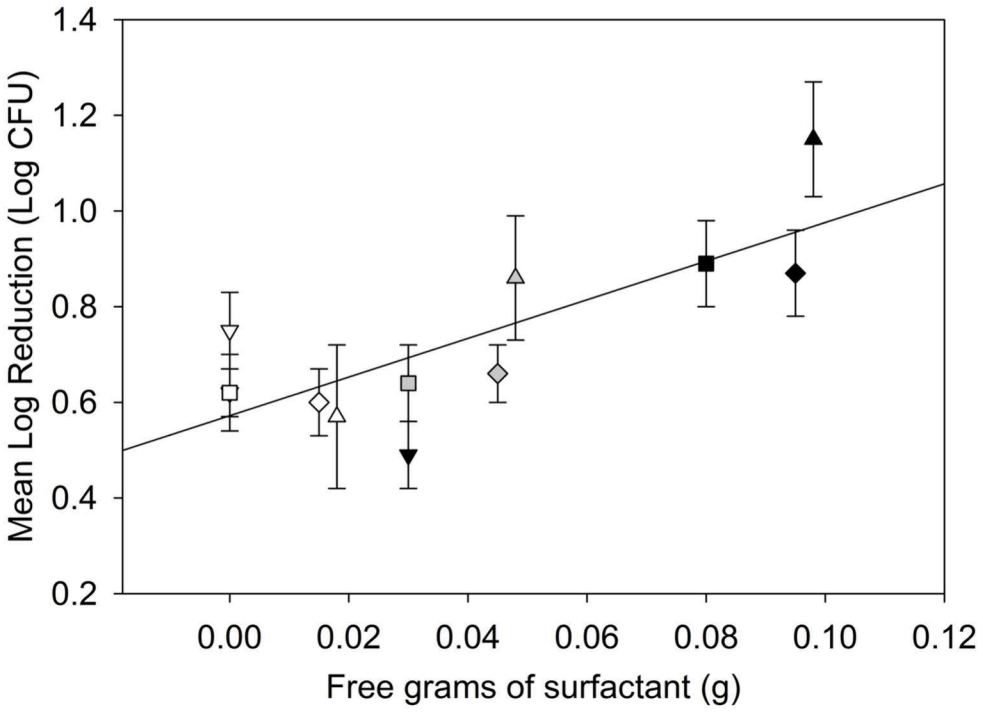


472

473

474 **Figure 2.** Correlation between free grams of surfactant (i.e. surfactant available to
475 form micelles) and mean log reduction. Surfactants in solution that were below the
476 critical micelle reported as zero free grams of surfactant. Open shapes are 2%
477 solutions, solid gray are 5% solutions, and solid black shapes are 10% solutions.
478 Sodium lauryl sulfate (△), Sodium stearoyl lactylate (▽), Poloxamer 407 (◇),
479 Sorbitan monostearate (□).

Fig 2



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