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(54) **Title**: BAR SOAP COMPOSITION

(57) **Abstract**: A bar soap composition provides an effective antimicrobial benefit against pathogens such as gram positive / gram negative bacteria and viruses. The bar soap compositions comprises: a) up to 10%wt. of a non-quaternary ammonium based germicidal compound, b) at least 45wt% of salts of C_{12}, C_{14},C_{16} and C_{18} saturated fatty acids, c) at least 20wt% water, d) up to 10%wt. of secondary alkane sulfonate. The bar soap is substantially free of silicate based fillers.

BAR SOAP COMPOSITION

The present invention relates to bar soap compositions which have multi-purpose functionality and are particularly useful in personal care applications, e.g. topical skin care, cleansing, which bar soap compositions exhibit an appreciable antimicrobial benefit.

Bar soaps are amongst the oldest forms of personal cleansing products. They are relatively easy to produce, as they are the form of solid bars or cakes require the simplest of packaging, typically boast long shelf storage lives, and of course are effective in providing a cleaning benefit. Many variations of such bar soaps are also known, and also widely available are bar soaps which additionally boasts an antimicrobial benefit. These are generally provided by the addition of known antimicrobial constituents, such as those based on antimicrobial free metal ions (e.g. Ag^{+}, Cu^{2+}, Zn^{2+}), phenolic antimicrobial compounds (e.g. TRICLOSAN, PCMX, TCC), non-phenolic antimicrobial compounds (e.g. certain quaternary ammonium salts) which independently of the bar soap composition provided antimicrobial benefit. The addition of these antimicrobial constituents, although well known to be effective, are also facing increasing scrutiny from regulatory agencies, and additionally require added material handling and costs during the production process of bar soaps containing such constituents.

According to a first aspect of the present invention there is provided bar soap composition providing an effective antimicrobial benefit against pathogens such as gram positive / gram negative bacteria and viruses, which bar soap compositions comprises:

a) up to 10%wt. of a non-quaternary ammonium based germicidal compound,

b) at least 45wt% of salts of C_{12}, C_{14} ,C_{16} and C_{18} saturated fatty acids,

c) at least 20wt% water,

d) up to 10%wt. of secondary alkane sulfonate,

which is substantially free of silicate based fillers.

The composition is preferably used in a cosmetic cleaning operation.

Thus according to a second aspect of the present invention there is provided a method for providing a germicidal benefit to a topical surface, especially a dermal surface, the method comprising the step of: contacting a topical surface upon which the presence of one or more undesired pathogens, preferably bacteria, are known or suspected, with a bar soap composition according to a first aspect of the invention.

The composition may be used in other cleaning operations.

Thus according to a third aspect of the present invention there is provided a method for providing a germicidal benefit to a topical surface, especially an inanimate surface, such as a fabric or a hard surface, e.g. dishware/cutlery, the method comprising the step of: contacting a topical surface upon which the presence of one or more undesired pathogens, preferably bacteria, are known or suspected, with a bar soap composition according to a first aspect of the invention.

In this way the invention provides a multi-use soap bar; thus saving the consumer the expense of having to purchase multiple soap bars for separate cleaning roles.

It is to be expressly understood that as used herein, “bar soap composition” refers to a composition which may, subject to appropriate processing conditions (e.g. compression) may be formed into a generally rigid, self-supporting solid bar soap, and references regarding the identity of constituents and weight percentages of a bar soap composition are similarly applicable to bar soaps formed therefrom.

The composition / method of the present invention provide as a primary technical benefit the reduction of undesired microorganisms, particularly in the reduction of both gram positive and gram negative microorganisms, while at the same time hi providing secondary benefits including the skin conditioning and/or skin cleansing.

Moreover it is been found that the composition of the present invention (and the method thereof) provide an especially mild soap formulation with low irritation effects, yet still providing the usual benefits of a soap formulation including an anti-microbial benefit. Thus repeated use for hygienic cleansing is possible without any of the usual side effects thereof. The particular mildness of the composition makes the composition especially suitable for use with babies / children.

Additionally it has been seen that the formulation displays excellent rinsibility characteristics yet with beneficial foam characteristics. In other words the formulation of the invention foams excellently when in use but said foam diminishes quickly allowing effective rinsing with low water usage.

Further ancillary benefits may be provided by the presence of one or more for the optional constituents which may be included in formulations or compositions according to the present intervention.

In more detail it has been observed that the bar soap composition is characterized in exhibiting at least a 1.5 log 10 reduction of Klebsiella pneumonia, S. aureus and E. coli when tested according to the standardized test protocols of ASTM E l 053 Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces

These aspects and advantages of the invention are discussed in more detail hereinafter, particularly are discussed in reference to one or more of the examples set forth below.

The soap constituent comprises at least 55% but preferably, is at least (in order of increasing preference, in %) 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 94.5, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, and 99.5% of salts of C_{12}, C_{14}, $C_{16}$and C_{18} saturated fatty acids.

The bar soap compositions of the invention may additionally include one or more anionic, nonionic, amphoteric or zwitterionic surfactants, particularly where such are provided to increase the production of foam or lather when the bar soap is used in a manual cleaning operation, e.g. washing of the hands, body or hair. Such surfactants are distinguished from the soap constituent described herein. Such are frequently referred to as synthetic surfactants, or "syndets" as they are distinguished from the fatty acid based soaps (frequently supplied as "soap noodles") which are the major constituent of the present invention. By way of non-limiting example, such include anionic surfactants which may be used in this capacity in the bar soaps include one or more of: alcohol sulfates and sulfonates, alcohol phosphates and phosphonates, alkyl ester sulfates, alkyl diphenyl ether sulfonates, alkyl sulfates, alkyl ether sulfates, sulfate esters of an alkylphenoxy polyoxyethylene ethanol, alkyl monoglyceride sulfates, alkyl sulfonates, alkyl ether sulfates, alpha-olefin sulfonates, beta-alkoxy alkane sulfonates, alkyl ether sulfonates, ethoxylated alkyl sulfonates, alkylaryl sulfonates, alkylaryl sulfates, alkyl monoglyceride sulfonates, alkyl carboxylates, alkyl ether carboxylates, alkyl alkoxy carboxylates having 1 to 5 moles of ethylene oxide, alkylpolyglycolethersulfates (containing up to 10 moles of ethylene oxide), sulfosuccinates, octoxynol or nonoxynol phosphates, taurates, fatty taurides, fatty acid amide polyoxyethylene sulfates, acyl glycerol sulfonates, fatty oleyl glycerol sulfates, alkyl phenol ethylene oxide ether sulfates, paraffin sulfonates, alkyl phosphates, isethionates (e.g. sodium cocoyl/lauroyl isethionate), N-acyl taurates, alkyl succinamates and sulfosuccinates, alkylpolysaccharide sulfates, alkylpolyglucoside sulfates, alkyl polyethoxy carboxylates, and sarcosinates or mixtures thereof.

More preferably the secondary alkane sulfonate is present in an amount of up to 8wt%, more preferably up to 6wt%, more preferably up to 5wt%, and most preferably up to 3wt%,

More preferably the secondary alkane sulfonate is present in an amount of greater than 0.1wt%, more preferably greater than 0.2wt%, more preferably greater than 0.3wt%, more preferably greater than 0.5wt%, more preferably greater than 1wt%, and most preferably greater than 1.5wt%.

Preferably the secondary alkane sulfonate comprises a C_6-C_{22} or secondary alkane sulfonate. A preferred example of which is available from Weylchem under the trade name of Hostapur SAS.

Further examples of anionic surfactants include water soluble salts or acids of the formula $(ROSO_3)_xM$ or $(RSO_3)_xM$ wherein R is preferably a C_6-C_{24} hydrocarbyl, preferably an alkyl or hydroxyalkyl having a C_{10}-C_{20} alkyl component, more preferably a C_{12}-C_{18} alkyl or hydroxyalkyl, and M is H or a mono-, di- or tri-valent cation, e.g. an alkali metal cation (e.g. sodium, potassium, lithium), or ammonium or substituted ammonium (e.g. methyl-, dimethyl-, and trimethyl ammonium cations and quaternary ammonium cations, such as tetramethyl-ammonium and dimethyl piperdinium cations and quaternary ammonium cations derived from alkylamines such as ethylamine, diethylamine, triethylamine, and mixtures thereof, and the like) and x is an integer, preferably 1 to 3, most preferably 1.

Further examples of anionic surfactants include alkyl-diphenyl-ethersulphonates and alkyl-carboxylates. Other anionic surfactants are C_6-C_{20} linear alkylbenzenesulfonates, C_6-C_{22} primary or alkanesulfonates, C_6-C_{24} olefinsulfonates, sulfonated polycarboxylic acids prepared by sulfonation of the pyrolyzed product of alkaline earth metal citrates, C_6-C_{24} alkylpolyglycolethersulfates , alkyl ester sulfates such as $C_{14\text{-}16}$ methyl ester sulfates; acyl glycerol sulfonates, fatty oleyl glycerol sulfates, alkyl phenol ethylene oxide ether sulfates, paraffin sulfonates, alkyl phosphates, isethionates such as the acyl isethionates, N-acyl taurates, alkyl succinamates and sulfosuccinates, monoesters of sulfosuccinate (especially saturated and unsaturated C_{12}-C_{18} monoesters) diesters of sulfosuccinate (especially saturated and unsaturated C_6-C_{14} diesters), acyl sarcosinates, sulfates of alkylpolysaccharides such as the sulfates of alkylpolyglucoside (the nonionic nonsulfated compounds being described below), branched primary alkyl sulfates, alkyl polyethoxy carboxylates such as those of the formula $RO(CH_2CH_2O)_kCH_2COO^-M^+$ wherein R is a C_8-C_{22} alkyl, k is an integer from 0 to 10, and M is a soluble salt-forming cation.

When present, such one or more anionic surfactants may be present in any effective amount, and advantageously comprise up to about 20%wt. of the bar soap compositions of which they form a part.

Non-limiting examples of nonionic surfactants which may be used in the bar soaps include one or more of: alkyl glucosides, alkyl polyglucosides, polyhydroxy fatty acid amides, alkoxylated fatty acid esters, amine oxides, and mixtures thereof. Further nonionic surfactants include almost any hydrophobic compound having a carboxy, hydroxy, amido, or amino group with a free hydrogen attached to the nitrogen which can be condensed with alkylene oxide (e.g. ethylene oxide, propylene oxide) or with the polyhydration product thereof, polyethylene glycol, to form a water soluble nonionic surfactant compound. Further, the length of the polyethenoxy hydrophobic and hydrophilic elements may various. Exemplary nonionic compounds include the polyoxyethylene ethers of alkyl aromatic hydroxy compounds, e.g. alkylated polyoxyethylene phenols, polyoxyethylene ethers of long chain aliphatic alcohols, the polyoxyethylene ethers of hydrophobic propylene oxide polymers, and the higher alkyl amine oxides.

Examples of nonionic surfactants include primary and secondary linear and branched alcohol ethoxylates, such as those based on C_6-C_{18} alcohols which further include an average of from 2 to 80 moles of ethoxylation per mole of alcohol. Examples include the Genapol® series of linear alcohol ethoxylates from Clariant Corp. Further nonionic surfactants include secondary C_{12}-C_{15} alcohol ethoxylates, including those which have from about 3 to about 10 moles of ethoxylation. Such are available in the Tergitol® series of nonionic surfactants (Dow Chemical, Midland, MI), particularly those in the Tergitol® "15-S-"series. Still further examples of suitable nonionic surfactants for use as the (b) at least one nonionic surfactant include which may be advantageously included in the inventive compositions are alkoxy block copolymers, and in particular, compounds based on ethoxy/propoxy block copolymers. Polymeric alkylene oxide block copolymers include nonionic surfactants in which the major portion of the molecule is made up of block polymeric C_2-C_4 alkylene oxides. Such nonionic surfactants, while preferably built up from an alkylene oxide chain starting group, and can have as a starting nucleus almost any active hydrogen containing group including, without

limitation, amides, phenols, thiols and secondary alcohols. Such are available in the Pluronic® series of block copolymer surfactants (ex. BASF). Other suitable non-ionic surfactants include alkoxylated alcohols (including propoxylation and butoxylation) – e.g. Plurafac series from BASF.

Examples of alkylpolyglycoside compounds include those which include alkyl monoglycosides and polyglycosides which may be prepared generally by reacting a monosaccharide, or a compound hydrolyzable to a monosaccharide with an alcohol such as a fatty alcohol in an acid medium. Exemplary alkyl glycoside surfactants alkyl glycoside surfactants suitable for use in the bar soaps of the present invention may be represented by formula (II) below:

$$RO\text{-}(R_1O)_y\text{-}(G)_xZ_b \qquad (II)$$

wherein:

R is a monovalent organic radical containing from about 6 to about 30, preferably from about 8 to about 18 carbon atoms;

R_1 is a divalent hydrocarbon radical containing from about 2 to about 4 carbon atoms;

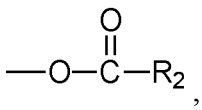
O is an oxygen atom;

y is a number which has an average value from about 0 to about 1 and is preferably 0;

G is a moiety derived from a reducing saccharide containing 5 or 6 carbon atoms; and

x is a number having an average value from about 1 to 5 (preferably from 1.1 to 2);

Z is O_2M^1,

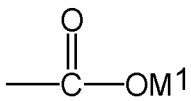


,

$O(CH_2)$, CO_2M^1, OSO_3M^1, or $O(CH_2)SO_3M^1$; R_2 is $(CH_2)CO_2M^1$ or $CH{=}CHCO_2M^1$;

(with the proviso that Z can be O_2M^1 only if Z is in place of a primary hydroxyl group in which the primary hydroxyl-bearing carbon atom,

$—CH_2OH$, is oxidized to form a



group);

b is a number of from 0 to 3x+1 preferably an average of from 0.5 to 2 per glycosal group;

p is 1 to 10,

M^1 is H^+ or an organic or inorganic cation, such as, for example, an alkali metal, ammonium, monoethanolamine, or calcium.

As defined in Formula II above, R is generally the residue of a fatty alcohol having from about 8 to 30 and preferably 8 to 18 carbon atoms. Examples of such alkylglycosides as described above include, for example, APG® 225 which is described as being an alkyl polyglycoside in which the alkyl group contains 8 to 10 carbon atoms and having an average degree of polymerization of 1.7, APG® 325 CS GLYCOSIDE which is described as being a 50% C_9-C_{11} alkyl polyglycoside, also commonly referred to as D-glucopyranoside, Glucopon® 425, described to be an alkyl polyglycoside in which the alkyl group contains 8 to 16 carbon atoms and having an average degree of polymerization of 1.48, Glucopon® 625 CS which is described as being a 50% C_{10}-C_{16} alkyl polyglycoside, also commonly referred to as a D-glucopyranoside, (available from Cognis Corp., Ambler PA), Plantaren® 2000, described as being an alkyl polyglycoside in which the alkyl group contains 8 to 16 carbon atoms and having an average degree of polymerization of 1.4, and Plantaren® 1300, described to be an alkyl polyglycoside in which the alkyl group contains 12 to 16 carbon atoms and having an average degree of polymerization of 1.6.

Further, and sometimes preferred nonionic surfactants which may be used in the bar soaps of the invention include certain alkanolamides including monoethanolamides and diethanolamides, particularly fatty monoalkanolamides and fatty dialkanolamides. Commercially available monoethanol amides and diethanol amides include those marketed under the trade names Alakamide® and Cyclomide® by Rhône-Poulenc Co., (Cranbury, NJ).

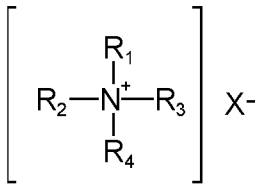
The bar soap compositions of the invention may include one or more sucrose ester based nonionic surfactants. Such are compounds which consist largely of sucrose mono- and di-esters of the natural fatty acids having 12 to 20 carbon atoms and preferably those having 16

to 20 carbon atoms. By way of non-limiting example, such include sucrose cocoate, sucrose dilaurate, sucrose distearate, sucrose laurate, sucrose myristate, sucrose oleate, sucrose palmitate, sucrose polylaurate, sucrose polylinoleate, sucrose polyoleate, sucrose polystearate, sucrose stearate, sucrose tetrastearate, sucrose tribehenate, sucrose tristearate or any combination thereof. Of these, preferred are sucrose cocoate and sucrose laurate, of which sucrose cocoate is particularly preferred. Whereas a mixture of sucrose ester based nonionic surfactants may be used, in certain particularly preferred embodiments it is preferred that the predominant sucrose ester based nonionic surfactant present is sucrose cocoate. In certain preferred embodiments, sucrose cocoate comprises at least 60%wt, and in order of increasing preference at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% and 100% by weight of the sucrose ether based nonionic surfactant constituent. When present the one or more sucrose ester based nonionic surfactants comprise from 0.001%wt. to about 3%wt., but more preferably from 0.05%wt. to about 1%wt. of bar soap compositions.

Exemplary useful amphoteric and zwitterionic surfactants include one or more of: alkyl betaines, alkyl amidobetaines, aminopropionates, aminoglycinates, imidazolinium betaines and sulfobetaines. Alkyl betaines are known surfactants which are mainly produced by carboxyalkylation, preferably carboxymethylation of aminic compounds. Typical examples are the carboxymethylation products of hexyl methyl amine, hexyl dimethyl amine, octyl dimethyl amine, decyl dimethyl amine, dodecyl methyl amine, dodecyl dimethyl amine, dodecyl ethyl methyl amine, $C_{12/14}$ cocoalkyl dimethyl amine, myristyl dimethyl amine, cetyl dimethyl amine, stearyl dimethyl amine, stearyl ethyl methyl amine, oleyl dimethyl amine, $C_{16/18}$ tallow alkyl dimethyl amine and technical mixtures thereof. Alkyl amidobetaines which represent carboxyalkylation products of amidoamines are also suitable. Typical examples are reaction products of fatty acids containing 6 to 22 carbon atoms, namely caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, palmitoleic acid, stearic acid, isostearic acid, oleic acid, elaidic acid, petroselic acid, linoleic acid, linolenic acid, elaeostearic acid, arachic acid, gadoleic acid, behenic acid and erucic acid and technical mixtures thereof, with N,N-dimethylaminoethyl amine, N,N-dimethylaminoproply amine, N,N-diethylaminoethyl amine and N,N-diethylaminoproply amine which are condensed with sodium chloroacetate.

When present, one or more of such anionic, nonionic, amphoteric or zwitterionic surfactants may be included in any effective amount. When present, such one or more said surfactants comprise about 0.1 – 25%wt. of the bar soap of which they form a part.

The bar soap may additionally include a quaternary ammonium based germicidal compound which independently provides an antimicrobial benefit. Such include quaternary ammonium compounds and salts thereof, which may be characterized by the general structural formula:

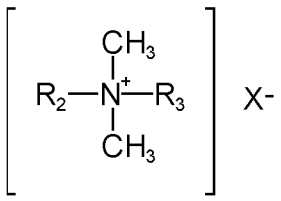


where at least one of R_1, R_2, R_3 and R_4 is a alkyl, aryl or alkylaryl substituent of from 6 to 26 carbon atoms, and the entire cation portion of the molecule has a molecular weight of at least 165. The alkyl substituents may be long-chain alkyl, long-chain alkoxyaryl, long-chain alkylaryl, halogen-substituted long-chain alkylaryl, long-chain alkylphenoxyalkyl, arylalkyl, etc. The remaining substituents on the nitrogen atoms other than the abovementioned alkyl substituents are hydrocarbons usually containing no more than 12 carbon atoms. The substituents R_1, R_2, R_3 and R_4 may be straight-chained or may be branched, but are preferably straight-chained, and may include one or more amide, ether or ester linkages. The counterion X may be any salt-forming anion which permits for the solubility or miscibility of the quaternary ammonium complex within the treatment composition.

Exemplary quaternary ammonium salts within the above description include the alkyl ammonium halides such as cetyl trimethyl ammonium bromide, alkyl aryl ammonium halides such as octadecyl dimethyl benzyl ammonium bromide, N-alkyl pyridinium halides such as N-cetyl pyridinium bromide, and the like. Other suitable types of quaternary ammonium salts include those in which the molecule contains either amide, ether or ester linkages such as octyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride, N-(laurylcocoaminoformylmethyl)-pyridinium chloride, and the like. Other very effective types

of quaternary ammonium compounds which are useful as germicides include those in which the hydrophobic radical is characterized by a substituted aromatic nucleus as in the case of lauryloxyphenyltrimethyl ammonium chloride, cetylaminophenyltrimethyl ammonium methosulfate, dodecylphenyltrimethyl ammonium methosulfate, dodecylbenzyltrimethyl ammonium chloride, chlorinated dodecylbenzyltrimethyl ammonium chloride, and the like.

Preferred quaternary ammonium compounds which exhibit an microbicidal effect, viz., act as germicides, and which are useful in the practice of the present invention include those which have the structural formula:



wherein R_2 and R_3 are the same or different C_8-C_{12}alkyl, or R_2 is C_{12-16}alkyl, C_{8-18}alkylethoxy, C_{8-18}alkylphenolethoxy and R_3 is benzyl, and X is a halide, for example chloride, bromide or iodide, a saccharinate counterion or is a methosulfate anion. The alkyl groups recited in R_2 and R_3 may be straight-chained or branched, but are preferably substantially linear.

Particularly useful quaternary ammonium compounds useful in the present inventive compositions include materials which include a single quaternary compound, as well as mixtures of two or more different quaternary compounds. Such useful quaternary compounds are available under the BARDAC®, BARQUAT®, HYAMINE®, LONZABAC®, and ONYXIDE® trademarks, which are more fully described in, for example, *McCutcheon's Functional Materials* (Vol. 2), North American Edition, 1998, as well as the respective product literature from the suppliers identified below. Such include, for example: BZT, which is described to be benzethonium chloride (*N*-benzyl-*N*,*N*-dimethyl-2-{2-[4-(2,4,4-trimethylpentan-2-yl)phenoxy]ethoxy}ethanaminium chloride); BARDAC® 205M which is described to be a liquid containing alkyl dimethyl benzyl ammonium chloride; octyl decyl dimethyl ammonium chloride; didecyl dimethyl ammonium chloride, and dioctyl dimethyl

ammonium chloride (50% active) (also available as 80% active (BARDAC® 208M)); BARDAC® 2050 which is described to be a combination of octyl decyl dimethyl ammonium chloride/didecyl dimethyl ammonium chloride, and dioctyl dimethyl ammonium chloride (50% active) (also available as 80% active (BARDAC® 2080)); BARDAC ® 2250 which is described to be didecyl dimethyl ammonium chloride (50% active); BARDAC® LF (or BARDAC® LF-80), described as being based on dioctyl dimethyl ammonium chloride (BARQUAT® MB-50, MX-50, OJ-50 (each 50% liquid) and MB-80 or MX-80 (each 80% liquid) are each described as an alkyl dimethyl benzyl ammonium chloride; BARDAC® 4250 and BARQUAT® 4250Z (each 50% active) or BARQUAT® 4280 and BARQUAT 4280Z (each 80% active) are each described as alkyl dimethyl benzyl ammonium chloride/alkyl dimethyl ethyl benzyl ammonium chloride. Also, HYAMINE® 1622, described as diisobutyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride (50% solution); HYAMINE® 3500 (50% actives), described as alkyl dimethyl benzyl ammonium chloride (also available as 80% active (HYAMINE® 3500-80)); and HYMAINE® 2389 described as being based on methyldodecylbenzyl ammonium chloride and/or methyldodecylxylene-bis-trimethyl ammonium chloride. (BARDAC®, BARQUAT® and HYAMINE® are presently commercially available from Lonza, Inc., Fairlawn, New Jersey). BTC® 50 NF (or BTC® 65 NF) is described to be alkyl dimethyl benzyl ammonium chloride (50% active); BTC® 99 is described as didecyl dimethyl ammonium chloride (50% active); BTC® 776 is described to be myrisalkonium chloride (50% active); BTC® 818 is described as being octyl decyl dimethyl ammonium chloride, didecyl dimethyl ammonium chloride, and dioctyl dimethyl ammonium chloride (50% active) (available also as 80% active (BTC® 818-80%)); BTC® 824 and BTC® 835 are each described as being of alkyl dimethyl benzyl ammonium chloride (each 50% active); BTC® 885 is described as a combination of BTC® 835 and BTC® 818 (50% active) (available also as 80% active (BTC® 888)); BTC® 1010 is described as didecyl dimethyl ammonium chloride (50% active) (also available as 80% active (BTC® 1010-80)); BTC® 2125 (or BTC® 2125 M) is described as alkyl dimethyl benzyl ammonium chloride and alkyl dimethyl ethylbenzyl ammonium chloride (each 50% active) (also available as 80% active (BTC® 2125 80 or BTC® 2125 M)); BTC® 2565 is described as alkyl dimethyl benzyl ammonium chlorides (50% active) (also available as 80% active (BTC® 2568)); BTC® 8248 (or BTC® 8358) is described as alkyl dimethyl benzyl ammonium chloride (80% active) (also

available as 90% active (BTC® 8249)); ONYXIDE® 3300 is described as n-alkyl dimethyl benzyl ammonium saccharinate (95% active). (BTC® and ONYXIDE® are presently commercially available from Stepan Company, Northfield, Illinois.).

The cationic quaternary ammonium compounds are preferably non-polymeric and/or non-oligomeric cationic surfactant compounds, and are thus distinguishable from the "Polyquat" polymers known to the art.

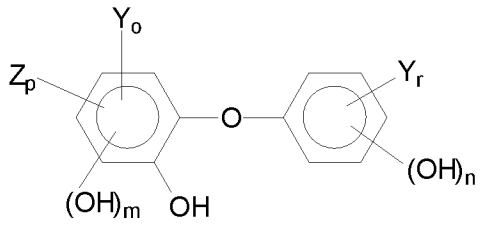
When present in a bar soap composition, the least one cationic quaternary ammonium compound(s) may be present in any effective amount, but generally need not be present in amounts in excess of about 10%wt. based on the total weight of the animate surface treatment composition of which it forms a part. Preferably, when present, the germicidal quaternary ammonium compound(s) may be present in the inventive compositions in amounts of from about 0.001 %wt. to up to about 10%wt., very preferably about 0.01-8%wt., more preferably in amounts of between about 0.01-2%wt., and most preferably from about 0.01 - 1%wt. It is particularly advantageous that the preferred germicidal cationic quaternary ammonium compound(s) are present in amounts of at least about 200 parts per million (ppm), preferably in amounts of from about 1 ppm to 10,000 ppm, preferably from about 50 ppm to 2000 ppm, more preferably in amounts of from about 100 ppm to 1,000 ppm.

The bar soap compositions may additionally include a non-quaternary ammonium based germicidal compound. Non-limiting examples of these compounds include: benzoyl peroxide, pyrithiones (especially zinc pyrithione which is also known as ZPT), dimethyldimethylol hydantoin (Glydant), methylchloroisothiazolinone/methylisothiazolinone (Kathon CG), sodium sulfite, sodium bisulfite, imidazolidinyl urea (Germall 115), diazolidinyl urea (Germaill II), benzyl alcohol, 2-bromo-2-nitropropane-1,3-diol (Bronopol), formalin (formaldehyde), iodopropenyl butylcarbamate (Polyphase P100), chloroacetamide, methanamine, methyldibromonitrile glutaronitrile (1,2-Dibromo-2,4-dicyanobutane or Tektamer), glutaraldehyde, 5-bromo-5-nitro- 1,3-dioxane (Bronidox), phenethyl alcohol, o-phenylphenol/sodium o-phenylphenol, sodium hydroxymethylglycinate (Suttocide A), polymethoxy bicyclic oxazolidine (Nuosept C), dimethoxane, thimersal dichlorobenzyl

alcohol, captan, chlorphenenesin, dichlorophene, chlorbutanol, glyceryl laurate, halogenated diphenyl ethers like 2,4,4-trichloro-2-hydroxy-diphenyl ether (Triclosan or TCS), 2,2-dihydroxy-5,5-dibromo-diphenyl ether, phenolic compounds like phenol, 2-methyl phenol, 3-methyl phenol, 4-methyl phenol, 4-ethyl phenol, 2,4-dimethyl phenol, 2,5-dimethyl phenol, 3,4-dimethyl phenol, 2,6-dimethyl phenol, 4-n-propyl phenol, 4-n-butyl phenol, 4-n-amyl phenol, 4-tert-amyl phenol, 4-n-hexyl phenol, 4-n-heptyl phenol, mono- and poly-alkyl and aromatic halophenols such as p-chlorophenol, methyl p-chlorophenol, ethyl p-chlorophenol, n-propyl p-chlorophenol, n-butyl p-chlorophenol, n-amyl p-chlorophenol, sec-amyl p-chlorophenol, n-hexyl p-chlorophenol, cyclohexyl p-chlorophenol, n-heptyl p-chlorophenol, n-octyl p-chlorophenol, o-chlorophenol, methyl o-chlorophenol, ethyl o-chlorophenol, n-propyl o-chlorophenol, n-butyl o-chlorophenol, n-amyl o-chlorophenol, tert-amyl o-chlorophenol, n-hexyl o-chlorophenol, n-heptyl o-chlorophenol, o-benzyl p-chlorophenol, o-benzyl-m-methyl p-chlorophenol, o-benzyl-m, m-dimethyl p-chlorophenol, o-phenylethyl p-chlorophenol, o-phenylethyl-m-methyl p-chlorophenol, 3-methyl p-chlorophenol, 3,5-dimethyl p-chlorophenol, 6-ethyl-3-methyl p-chlorophenol, 6-n-propyl-3-methyl p-chlorophenol, 6-iso-propyl-3-methyl p-chlorophenol, 2-ethyl-3,5-dimethyl p-chlorophenol, 6-sec-butyl-3-methyl p-chlorophenol, 2-iso-propyl-3,5-dimethyl p-chlorophenol, 6-diethylmethyl-3-methyl p-chlorophenol, 6-iso-propyl-2-ethyl-3-methyl p-chlorophenol, 2-sec-amyl-3,5-dimethyl p-chlorophenol 2-diethylmethyl-3,5-dimethyl p-chlorophenol, 6-sec-octyl-3-methyl p-chlorophenol, p-chloro-m-cresol, p-bromophenol, methyl p-bromophenol, ethyl p-bromophenol, n-propyl p-bromophenol, n-butyl p-bromophenol, n-amyl p-bromophenol, sec-amyl p-bromophenol, n-hexyl p-bromophenol, cyclohexyl p-bromophenol, o-bromophenol, tert-amyl o-bromophenol, n-hexyl o-bromophenol, n-propyl-m,m-dimethyl o-bromophenol, 2-phenyl phenol, 4-chloro-2-methyl phenol, 4-chloro-3-methyl phenol, 4-chloro-3,5-dimethyl phenol, 2,4-dichloro-3,5-dimethylphenol, 3,4,5,6-terabromo-2-methylphenol, 5-methyl-2-pentylphenol, 4-isopropyl-3-methylphenol, para-chloro-meta-xylenol ("PCMX"), dichloro meta xylenol, chlorothymol, 5-chloro-2-hydroxydiphenylmethane, resorcinol and its derivatives including methyl resorcinol, ethyl resorcinol, n-propyl resorcinol, n-butyl resorcinol, n-amyl resorcinol, n-hexyl resorcinol, n-heptyl resorcinol, n-octyl resorcinol, n-nonyl resorcinol, phenyl resorcinol, benzyl resorcinol, phenylethyl resorcinol, phenylpropyl resorcinol, p-chlorobenzyl resorcinol, 5-chloro 2,4-dihydroxydiphenyl methane, 4-chloro 2,4-

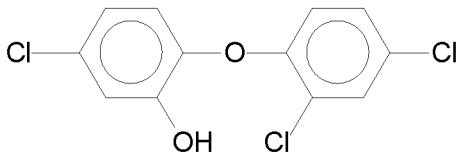
dihydroxydiphenyl methane, 5-bromo 2,4-dihydroxydiphenyl methane, and 4-bromo 2,4-dihydroxydiphenyl methane, bisphenolic compounds like 2,2-methylene bis (4-chlorophenol), 2,2-methylene bis (3,4,6-trichlorophenol), 2,2-methylene bis (4-chloro-6-bromophenol), bis (2-hydroxy-3,5-dichlorophenyl) sulphide, and bis (2-hydroxy-5-chlorobenzyl)sulphide, benzoic esters (parabens) like methylparaben, propylparaben, butylparaben, ethylparaben, isopropylparaben, isobutylparaben, benzylparaben, sodium methylparaben, and sodium propylparaben, halogenated carbanilides (e.g., 3,4,4-trichlorocarbanilides (Triclocarban or TCC), 3-trifluoromethyl-4,4-dichlorocarbanilide, 3,3,4-trichlorocarbanilide, etc.).

Of these, preferred are phenol based non-cationic microbicidals (antimicrobial constituents), especially those based on one or more phenolic compounds, particularly 2-hydroxydiphenyl compounds which may be exemplified by the following classes of compounds:



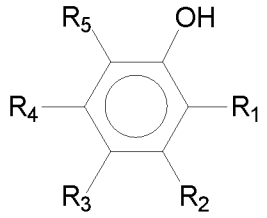
wherein Y is chlorine or bromine, Z is SO_2 H, NO_2, or C_1 -C_4 alkyl, r is 0 to 3, o is 0 to 3, p is 0 or 1, m is 0 or 1, and n is 0 or 1. In preferred embodiments, Y is chlorine or bromine, m is 0, n is 0 or 1, o is 1 or 2, r is 1 or 2, and p is 0, and according to especially preferred embodiments, Y is chlorine, m is 0, n is 0, o is 1, r is 2, and p is 0.

Particularly useful 2-hydroxydiphenyl compounds include those which may be represented by the structure:



which is commonly referred to as "TRICLOSAN" and which is presently commercially available from Ciba Specialty Chemicals Corp., as well as halogenated carbanilides, e.g., TCC.

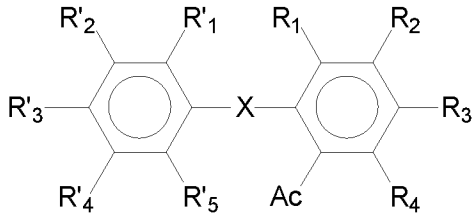
Further exemplary useful phenolic based antimicrobial constituents agents include 2,2'-hydroxy-5,5'-dibromo-diphenyl ether which may be represented by the structure:



wherein R_1 is hydro, hydroxy, C_1 -C_4 alkyl, chloro, nitro, phenyl, or benzyl; R_2 is hydro, hydroxy, C_1 -C_6 alkyl, or halo; R_3 is hydro, C_1 -C_6 alkyl, hydroxy, chloro, nitro, or a sulphur in the form of an alkali metal salt or ammonium salt; R_4 is hydro or methyl, and R_5 is hydro or nitro. Halo is bromo or, preferably, is chloro.

Specific examples of phenol derivatives include, but are not limited to, chlorophenols (o-, m-, p-), 2,4-dichlorophenol, p-nitrophenol, picric acid, xylenol, p-chloro-m-xylenol, cresols (o-, m-, p-), p-chloro-m-cresol, pyrocatechol, resorcinol, 4-n-hexylresorcinol, pyrogallol, phloroglucin, carvacrol, thymol, p-chlorothymol, o-phenylphenol, o-benzylphenol, p-chloro-o-benzylphenol, phenol, 4-ethylphenol, and 4-phenolsulfonic acid.

Still further useful phenol derivatives include those which may be represented by the structure:



wherein X is sulphur or a methylene group, R_1 and R'_1 are hydroxy, and R_2, R'_2, R_3, R'_3, R_4, R'_4, R_5, and R'_5, independent of one another, are hydro or halo. Specific, nonlimiting examples of diphenyl compounds are hexachlorophene, tetrachlorophene, dichlorophene, 2,3-dihydroxy-5,5'-dichlorodiphenyl sulfide, 2,2'-dihydroxy-3,3',5,5'-tetrachlorodiphenyl sulfide, 2,2'-dihydroxy-3,5',5,5', 6,6'-hexachlorodiphenyl sulfide, and 3,3'-dibromo-5,5'-dichloro-2,2'-

dihydroxydiphenylamine. Of the foregoing, a particularly useful phenol derivative is commonly referred to as triclocarban, or 3,4,4'-trichlorocarbanilide as well as derivatives thereto.

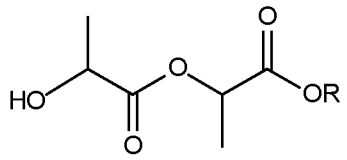
More preferably said non-quaternary ammonium based germicidal compound is TCC and/or PCMX.

When present in a bar soap composition, the least one non-quaternary ammonium based germicidal compound may be present in any effective amount, but generally need not be present in amounts in excess of about 10%wt. based on the total weight of the animate surface treatment composition of which it forms a part. Preferably, when present, the non-quaternary ammonium based germicidal compound(s) may be present in the inventive compositions in amounts of from about 0.001 %wt. to up to about 10%wt., very preferably about 0.01-8%wt., more preferably in amounts of between about 0.01-2%wt., and most preferably from about 0.01 - 1%wt. It is particularly advantageous that the preferred non-quaternary ammonium based germicidal compound(s) are present in amounts of at least about 200 parts per million (ppm), preferably in amounts of from about 1 ppm to 10,000 ppm, preferably from about 50 ppm to 2000 ppm, more preferably in amounts of from about 100 ppm to 1,000 ppm.

The bar soaps of the present invention may include still further constituents. Non-limiting examples of such further constituents are described herein.

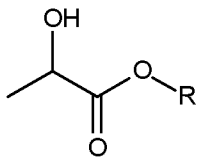
The bar soap compositions may include an effective amount of an anti-acne agent. Such may be any compound, composition or material which has been approved by the U.S. Food and Drug Administration for the topical treatment of acne. Examples of anti-acne agents include, but are not limited to, salicylic acid, benzoyl peroxide, sulphur, retinoic acid, candida bombicola/glucose/methyl rapeseedate ferment, peat water, resorcinol, silt, peat, permethin, azelaic acid, clindamycin, adapalene, erythromycin, sodium sulfacetamide, and combinations thereof. Of these, benzoyl peroxide is particularly preferred.

Further useful constituents include one or more alkyl lactates, which may themselves provide an antimicrobial benefit. Such include the reaction products of a C_8-C_{18} fatty alcohol with lactic acid. Preferred alkyl lactates include those represented by the following general structural formula (Ia):



(Ia)

in which R is a C_8-C_{18} alkyl moiety, preferably is a C_{10}-C_{14} alkyl moiety and especially preferably is predominantly (at least 85%, more preferably at least 90%, particularly preferably at least 95% and most preferably at least about 98%) of a C_{12} alkyl moiety. The alkyl moiety may be branched but is preferably substantially linear. A particularly preferred alkyl lactate conforming to formula (Ia) is lauryl lactyl lactate. Preferred alkyl lactates also include those represented by the following general structural formula (Ib):



(Ib)

in which R is a C_8-C_{18} alkyl moiety, preferably is a C_{10}-C_{14} alkyl moiety and especially preferably is predominantly (at least 85%, more preferably at least 90%, particularly preferably at least 95% and most preferably at least about 98%) of a C_{12} alkyl moiety. The alkyl moiety may be branched but is preferably substantially linear. A particularly preferred alkyl lactate conforming to formula (Ib) is lauryl lactyl lactate. Of course it is to be understood that other alkyl lactates not specifically encompassed by the compounds of formula (Ia) and/or (Ib) may also be utilized. When present such one or more alkyl lactates may be present in any effective amount, but advantageously comprise between about 0.001%wt. to about 3%wt., more preferably between about 0.05%wt. to about 0.5%wt. of a bar soap composition.

The soap bars may include one or more polyols. Such include compounds having two or more hydroxyl groups and which are highly water soluble, preferably freely soluble, in water. Non-limiting examples of suitable polyols include: relatively low molecular weight short chain polyhydroxy compounds such as glycerol and propylene glycol; sugars such as sorbitol, manitol, sucrose and glucose; carbohydrates such as starch (27% amylose and 73% amylopectin non-waxy starch) and modified carbohydrates such as hydrolyzed starch, dextrin and maltodextrin, and polymeric synthetic polyols such as polyalkylene glycols, for example polyoxyethylene glycol (PEG) and polyoxypropylene glycol (PPG).

More preferably the polyol is present in an amount of 1-30wt%, more preferably 5-25wt%, more preferably 10-25wt%, and most preferably about 18wt%.

Most preferably the polyol comprises starch.

Without wishing to be bound by theory the inventors have found that the starch plays an important role in maintaining the structural integrity of the formulation of the invention (particularly for such a high water containing formulation). It is postulated that this effect is achieved via an interaction between the starch and any ionic salt present in the formulation.

In use the starch has been found to have many beneficial effects including the formation of film on skin and also the provision of a moisturizing effect.

Of these said polyols, preferred are relatively low molecular weight compound which are either liquid or readily soluble in aqueous solutions, e.g., low molecular weight polyols and sugars. Particularly preferred polyols are glycerine, glycerol, sorbitol and their mixtures. Glycerine and glycerol are particularly preferred, as such may also provide benefits as humectants in the bar soaps.

When present, such one or more polyols may be included in minor but effective amounts, e.g., from about 0.001%wt. to about 0.5%wt., more preferably from about 0.1 -10%wt. and

especially preferably from about 0.5 – 9%wt. based on the total weight of the bar soap of which it forms a part.

The bar soap compositions of the invention may include one or more stearyl alkanoates, preferably one or more selected from stearyl caprylate, stearyl palmitate, stearyl stearate, stearyl behenate, and stearyl olivate. Of these, stearyl heptanoate is particularly preferred. Whereas a mixture of stearyl alkanoates may be used, in certain particularly preferred embodiments it is preferred that the predominant stearyl alkanoate present is stearyl heptanoate. In certain preferred embodiments, stearyl heptanoate comprises at least 60%wt, and in order of increasing preference at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% and 100% by weight of the stearyl alkanoates present. The overall content of the one or more stearyl alkanoates in the bar soap compositions is preferably from 0.001%wt. to about 5%wt., more preferably is from 0.05%wt. to about 0.8%wt.

One or more insoluble filler materials may also be present in the bar soap compositions. Advantageously such are provided as powders or comminuted particulates of aqueous insoluble materials such that due to their small size they are readily incorporated into the compositions from which the bar soaps are produced. These filler materials may be inorganic or organic or a combination as long as it is insoluble in water. The insoluble particles should not be perceived by a user of the bar soap as unduly abrasive or granular and advantageously such filler materials have an average particle of size less than 300 microns, more preferably less than 100 microns and most preferably less than 50 microns. Preferably the insoluble particles have a maximum particle size of 300 microns or less, preferably 200 microns or less.

Non-limiting examples of inorganic particulate materials include calcium carbonate. Calcium carbonate or as it is interchangeable referred to as “chalk” exists in three crystal forms: calcite, aragonite and vaterite. The natural morphology of calicite is rhombohedral or cuboidal, acicular or dendritic for aragonite and spheroidal for vaterite. Commercially, calcium carbonate or chalk is also known as precipitated calcium carbonate and is produced by a carbonation method in which carbon dioxide gas is bubbled through an aqueous

suspension of calcium hydroxide. In this process the crystal type of calcium carbonate is calcite or a mixture of calcite and aragonite.

Further non-limiting examples of suitable optional insoluble inorganic particulate materials include, , phosphates, insoluble sulfates such as sodium sulfate, and borates and as well as mixtures thereof.

The composition is substantially free of silicate based fillers.

Non-limiting examples of such materials include talc. Talc is a magnesium silicate mineral material, with a sheet silicate structure and a composition of $Mg_3Si_4(OH)_{22}$, and may be available in a hydrated form. Talc is considered hydrophobic as it is wetted by oil rather than water. Further non-limiting examples of suitable optional insoluble inorganic particulate materials include alumino silicates, silicates, and clays (e.g., kaolin, china clay) as well as mixtures thereof

Non-limiting examples of organic particulate materials include: insoluble polysaccharides such as highly cross linked or insolubilized starch (e.g., by reaction with a hydrophobe such as octyl succinate) and cellulose; synthetic polymers such as various polymer lattices and suspension polymers; insoluble soaps and mixtures thereof.

When present one or more of the foregoing insoluble filler materials may comprise up to about 20%wt. of the bar soap of which it forms a part, but advantageously, when present in included in an amount of from about 0.01%wt. to about 10%wt. Particularly preferred insoluble filler materials and amounts useful in the bar soaps of the invention are disclosed with reference to one or more of the Examples.

One or more soluble materials (which may serve as a filler) may also be present in the bar soap compositions. These soluble materials may preferably comprise ionic salts. Preferably these materials comprise one or more alkali metal (e.g. lithium, sodium, potassium), alkaline metal (e.g. magnesium, calcium), organic cation (such as ammonium) or transition metal (e.g.

iron, titanium, copper or zinc). Preferred anions for these salts include halide, nitrate, sulphate and carbonate.

Indeed it has been noted that soluble materials can act as structuring aids, aiding the structural integrity of the soap bar. Without wising to be bound by theory it is postulated that this effect occurs via electrolytic interaction.

The bar soap compositions may include one or more organosiloxane containing constituents, especially polysiloxane containing compounds which may provide a skin treatment benefit to an epidermal surface treated with the bar soap of the invention. Such materials are known per se, and are often interchangeably referred to as silicone emulsifiers. Such silicone emulsifiers include polydiorganosiloxanepolyoxyalkylene copolymers containing at least one polydiorganosiloxane segment and at least one polyoxyalkylene segment. The polyoxyalkylene segments may be bonded to the polydiorganosiloxane segments with silicon-oxygen-carbon bonds and/or with silicon-carbon bonds. The polydiorganosiloxane segments of consist essentially of siloxane units which are interlinked by Si-O-Si linkages and which have the formula:

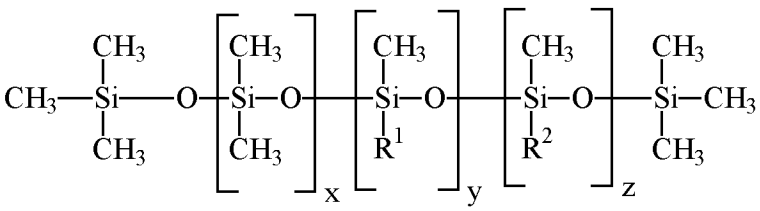
$$R_bSiO_{(4\{b\})/2}$$

The value of b may range from 0 to 3 for said siloxane units with the provision that there is an average of approximately 2, i.e. from 1.9 to 2.1 R radicals for every silicon in the copolymer. Suitable siloxane units thus include $R_3SiO_{1/2}$, $R_2SiO_{2/2}$, $RSiO_{3/2}$, and $SiO_{4/2}$ siloxane units taken in such molar amounts so that b has an average value of approximately 2 in the copolymer. Said siloxane units may be arranged in linear, cyclic and/or branched fashion. The R radicals may be any radical selected from the group consisting of methyl, ethyl, vinyl, phenyl, and a divalent radical bonding a polyoxyalkylene segment to the polydiorganosiloxane segment. At least 95 percent of all R radicals are methyl radicals; preferably there is at least one methyl radical bonded to each silicon atom in (d). Divalent R radicals preferably contain no more than 6 carbon atoms. Examples of divalent R radicals include --O--, $--C_mH_{2m}O--$, $--C_mH_{2m}$ -- and $--C_mH_{2m}CO_2$ -- where m is an integer greater than zero. Illustrative of the siloxane units that make up the polydiorganosiloxane segments are the following, where Me denotes methyl and

Q denotes said divalent R radical and bonded polyoxyalkylene segment: $R_3SiO_{1/2}$ units such as $Me_3SiO_{1/2}$, $Me_2(CH_2=CH)SiO_{1/2}$, $Me_2(C_6H_5)SiO_{1/2}$, $Me(C_6H_5)(CH_2=CH)SiO_{1/2}$, $Me_2(CH_3CH_2)SiO_{1/2}$, $Me_2QSiO_{1/2}$, $MeQ_2SiO_{1/2}$, $Q_3SiO_{1/2}$, $Q_2(CH_3CH_2)SiO_{1/2}$, and $Me(C_6H_5)(Q)SiO_{1/2}$; $R_2SiO_{2/2}$ units such as $Me_2SiO_{2/2}$, $Me(C_6H_5)SiO_{2/2}$, $Me(CH_2=CH)SiO_{2/2}$, $(C_6H_5)_2SiO_{2/2}$, $MeQSiO_{2/2}$, and $Q(C_6H_5)SiO_{2/2}$; $RSiO_{3/2}$ units such as $MeSiO_{3/2}$, $C_6H_5SiO_{3/2}$, $CH_2=CHSiO_{3/2}$, $CH_3CH_2SiO_{3/2}$ and $QSiO_{3/2}$; and $SiO_{4/2}$ units.

Volatile linear silicones including polydimethylsiloxane and dimethicones may also be present as silicone emulsifiers in compositions according to the invention.

Also useful as silicone emulsifiers in the inventive compositions are one or more compounds which may be represented by the structure:



wherein

R^1 represents a C_1-C_{30} straight chained, branched or cyclic alkyl group,

R^2 represents a moiety selected from:

$$—(CH_2)_n—O—(CH_2CHR^3O)_m—H$$

and

$$—(CH_2)_n—O—(CH_2CHR^3O)_m—(CH_2CHR^4O)_p—H$$

in which n represents an integer from about 3 to about 10, R3 and R4 are selected from hydrogen and C_1-C_6 straight chain, or branched chain alkyl groups with the proviso that R^3 and R^4 are not simultaneously the same, each of m, p, x and y are independently selected from integers of zero or greater, such that the molecule has a molecular weight of between about 200 to about 20,000,000 and wherein both m and p are not both simultaneously zero, and z is selected from integers of 1 or greater.

When present, one or more of the foregoing organosiloxane containing constituents may comprise up to about 5%wt. of the bar soap of which it forms a part, but advantageously, when present in included in an amount of from about 0.01%wt. to about 1.5%wt. In certain embodiments a organosiloxane containing constituent is necessarily present. Particularly preferred organosiloxane containing constituents and amounts useful in the bar soaps of the invention are disclosed with reference to one or more of the Examples

The bar soap compositions may include one or more optical modifying constituents, such as reflecting materials and pearlizing agents which provide a frequently desirable appearance to the bar soap. Such optical modifying constituents may be inorganic materials, such as one or more of: titanium dioxide, coated micas and other interference pigments; plate like mirror particles such as organic glitters. Further useful optical modifiers may be based on organic materials or compounds, such as one or more of latexes presently commercially available under the trademark ACUSOL (ex. Rohm & Haas Inc.) which are characterized by pH of about 2 to about 3, having approximately 40% solids in water, with particle size of about 0.1 to about 0.5 micron; styrene/polyvinylpyrrolidone co-polymers and styrene/acrylic emulsions, such as styrene/polyvinylpyrrolidone co-polymers available as POLECTRON 430 (ex. ISP Technologies, Inc.), as well as styrene/acrylamide emulsion such as OPULYN (ex. Rohm & Haas Inc.).

The bar soaps may include as optical modifying constituents one or more optical brighteners. By way of nonlimiting examples, such include 4,4'-diamino-2,2'-stilbenedisulfonic acids (flavonic acids), 4,4'-distyrylbiphenyls, methylumbelliferones, coumarins, dihydroquinolinones, 1,3-diarylpyrazolines, naphthalimides, benzoxazole, benzisoxazole and benzimidazole systems, and the pyrene derivatives substituted by heterocycles. Specific examples of such optical brighteners include those sold under the trade name TINOPAL (ex. Ciba) such and as TINOPAL CBS which is described to be disodium 2,2'-bis-(phenyl-styryl)disulphonate as well as TINOPAL DMS which is described to be disodium 4,4'bis-(2-morpholino-4-anilino-s-triazin-6-ylamino)stilbene disulphonate. Such optical brighteners may be included in useful amounts; exemplary useful amounts generally fall within the range on from 0.001%wt. to 0.1%wt.

When present, such optical modifying constituents are advantageously included in generally minor amounts such as from 0.001 - 1 %wt. but desirably are present in amounts from 0.01 – 0.75%wt. In certain preferred embodiments an optical modifying constituents is necessarily present in the bar soaps.

The bar soap compositions may include one or more fragrance materials which may be a one or more compounds which impart an olfactive effect from the bar soap. Exemplary fragrance materials may be based on natural and synthetic fragrances and most commonly are mixtures or blends of a plurality of such fragrances, optionally in conjunction with a carrier such as an organic solvent or a mixture of organic solvents in which the fragrances are dissolved, suspended or dispersed. Such may be natural fragrances, e.g., natural extracts of plants, fruits, roots, stems, leaves, wood extracts, e.g. terpineols, resins, balsams, animal raw materials, e.g., civet and beaver, as well as typical synthetic perfume compounds which are frequently products of the ester, ether, aldehyde, ketone, alcohol and hydrocarbon type, e.g., benzyl acetate, linalyl acetate, citral, citronellal, methyl cedryl ketone, eugenol, isoeugenol, geraniol, linalool, and Typically it is preferred to use mixtures of different perfume compounds which, together, produce an agreeable fragrance. Other suitable perfume oils are essential oils of relatively low volatility which are mostly used as aroma components. Examples are sage oil, camomile oil, clove oil, melissa oil, mint oil, cinnamon leaf oil, lime-blossom oil, juniper berry oil, vetiver oil, olibanum oil, galbanum oil, labolanum oil and lavendin oil.

In a preferred embodiment, the fragrance itself displays malodor control properties

In a preferred embodiment the fragrance itself displays anti-microbial properties. Preferred example of an anti-microbial compositions are disclosed in, for example, EP1776944, the contents of which are incorporated by reference. A preferred example of such an anti-microbial fragrance or flavor has the following composition.

Dihydrofamesol – 50wt%
Neroridol – 30wt%

Dodecanal – 5wt%

Nookatone – 5wt%

2-Methyl decanal – 5wt%

Trans-2-Undecanal – 5wt%

When present in a treatment composition, in accordance with certain of the preferred embodiments, the fragrance constituent may be present in any effective amount such that it can be discerned by a consumer of the composition, however is advantageously present in amounts of up to about 2%wt., preferably are present in amounts of from about 0.00001%wt. to about 1.25%wt. of the bar soap.

The bar soap compositions may include one or more colouring agents, such as one or more dyes and/or pigments, which may be present in effective amounts. Advantageously such one or more colouring agents are present in amounts of about 0.0001 – 1%wt. of the bar soap which include said one or more colouring agents.

The bar soap compositions may also optionally include a preservative constituent which is used to control the undesired where the microorganisms within the treatment composition is particularly in long-term storage and at elevated temperatures. Such are usually distinguished from the optional non-cationic compounds which provide an antimicrobial or germicidal discussed above, as preservative constituents typically are included in minor amounts which are effective in providing a useful benefit to regard spoilage or unwanted microbial growth in the bar soap itself, but are ineffective in providing a useful antimicrobial benefit when dissolved with water to form a washing solution and/or formed into a lather which washing solution and/or lather themselves provided a useful cleaning and/or antimicrobial benefit, particularly to treated dermal surfaces. Thus, such ancillary preservative constituents may be included in minor but effective amounts. Nonlimiting examples include one or more of parabens, including methyl parabens and ethyl parabens, glutaraldehyde, formaldehyde, 2-bromo-2-nitropropoane-1,3-diol, 5-chloro-2-methyl-4-isothiazolin-3-one, 2-methyl-4-isothiazoline-3-one, and mixtures thereof. One exemplary composition is a combination 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one where the amount of

either component may be present in the mixture anywhere from 0.001 to 99.99 weight percent, based on the total amount of the preservative. Further exemplary useful preservatives include those which are commercially including a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one marketed under the trademark KATHON® CG/ICP as a preservative composition presently commercially available from Rohm and Haas (Philadelphia, PA). Typically, when present, the preservative constituent is advantageously present in an amount from about 0.00001 - 0.5%wt. of the bar soap.

The bar soap compositions may include one or more antioxidants such as, for example, butylated hydroxytoluene (BHT). One or more antioxidants when present, are advantageously present in any effective amount, e.g., 0.00001% - 0.5%wt. of the bar soap.

The bar soap compositions may include one or more chelating agents. Exemplary useful chelating agents include those known to the art, including by way of non-limiting example; etidronic acids and aminopolycarboxylic acids and salts thereof wherein the amino nitrogen has attached thereto two or more substituent groups. Preferred chelating agents include acids and salts, especially the sodium and potassium salts of ethylenediaminetetraacetic acid, diethylenetriaminepentaacetic acid, N-hydroxyethylethylenediaminetriacetic acid, and of which the sodium salts of ethylenediaminetetraacetic acid may be particularly advantageously used. Such chelating may be included in generally minor amounts such as from about 0.001 - 0.5 %wt. based on the weight of the chelating agents and/or salt forms thereof.

The bar soap compositions may include one or more pH adjusting agents, (which may also be one or more pH buffers) which may be used to establish and/or maintain a desired pH of the compositions from which the bar soaps are formed, as well as to the bar soap itself. Essentially any material which may increase or decrease the pH of the bar soap composition is suitable as a pH adjusting agent. Suitable pH adjusting agents are one or more acids and/or bases whether such be based on organic and/or inorganic compounds or materials. By way of non-limiting example, pH adjusting agents include phosphorus containing compounds, monovalent and polyvalent salts such as of silicates, carbonates, and borates, certain acids and bases, tartrates and certain acetates. Further exemplary pH adjusting agents include mineral

acids, basic compositions, and organic acids, which are typically required in only minor amounts. Further exemplary and useful pH adjusting agents include monoalkanolamines, dialkanolamines, trialkanolamines, and alkylalkanolamines such as alkyl-dialkanolamines, and dialkyl-monoalkanolamines. Such may also function as detersive surfactants. The alkanol and alkyl groups are generally short to medium chain length, that is, from 1 to 7 carbons in length.

By way of further non-limiting example, pH buffering agents include the alkali metal phosphates, polyphosphates, pyrophosphates, triphosphates, tetraphosphates, silicates, metasilicates, polysilicates, carbonates, hydroxides, and mixtures of the same. Certain salts, such as the alkaline earth phosphates, carbonates, hydroxides, can also function as buffers. It may also be suitable to use as buffers such materials as aluminosilicates (zeolites), borates, aluminates and certain organic materials such as gluconates, succinates, maleates, citrates, and their alkali metal salts. When present, the one or more pH adjusting agents are included in amounts which are effective in establishing and/or maintaining the pH of a treatment composition at or desired pH value or within a range of pH values. Advantageously the one or more pH adjusting agents comprise from about 0.001 – 2.5%wt., preferably from about 0.01 – 1.5%wt. of the treatment composition of which the one or more pH adjusting agents form a part.

Optionally the bar soap compositions may include one or more skin benefit agents which may be used to promote an improved skin feel, or to improve skin health or appearance, or to promote hair health or appearance. Such include but are not limited to lipids such as cholesterol, ceramides, and pseudoceramides; sunscreens such as cinnamates; other types of exfoliant particles such as polyethylene beads, walnut shells, apricot seeds, flower petals and seeds, and inorganics such as silica, and pumice; additional emollients (skin softening agents) such as long chain alcohols and waxes e.g., lanolin; additional moisturizers; skin-toning agents; skin nutrients such as vitamins like Vitamin C, D and E and essential oils like bergamot, citrus unshiu, calamus, and the like; water soluble or insoluble extracts of avocado, grape, grape seed, myrrh, cucumber, watercress, calendula, elder flower, geranium, linden

blossom, amaranth, seaweed, gingko, ginseng, carrot; impatiens balsamina, camu camu, alpine leaf and other plant extracts such as witch-hazel, and mixtures thereof.

An amount of water is present in the bar soap and the bar soap compositions from which the bar soaps are formed. Such is in an amount of more than 20wt%, preferably more than 21wt%, more preferably more than 22wt%, more preferably more than 23wt%, and most preferably about 25wt% is provided to the remaining constituents of a bar soap composition. It is to be realized that in certain of the other constituents, a minor amount of water may be present and may thus be supplied to a bar soap composition from which a bar soap is made; such sources of water may be considered to be "added water" as defined herein.

The inventors have surprisingly found that the formulation of the invention displays a sufficient structural integrity even at such high water content: higher than is normally present in such a soap bar.

As noted previously, the bar soap compositions described above may be formed into bar soaps according to conventional production methods known to the art.

Advantageously the bar soaps are made by a process which involved both the intensive mixing or working of the soap mass while it is in a semi-solid plastic state and its forming into a cohesive mass by the process of extrusion. The intensive mixing can be accomplished by one or more unit operations known in the art which can include roller milling, refining, and single or multistage extrusion. Such processes work the bar soap composition preferably at a temperature of between about 20°C and about 70°C to form a homogeneous network of insoluble materials in a viscous liquid and/or liquid crystalline phase containing the lower melting, more soluble surfactants (e.g., soaps and other water soluble/dispersible materials). The extruded mass must be thermoplastic within the process temperature of extrusion which is generally between about 20° C and about 60°C, Thus, the bar soap composition must soften within this process temperature window but remain highly viscous, i.e., not softer excessively to form a sticky mass. The material must regain its structure and harden quickly as the temperature is lowered below its softening point. The softened mass although pliable must be

sufficiently viscous so that it does not stick to the surfaces of the extruder in order to be capable of conveyance by the extruder screws but not bend excessively when exiting the extruder as a billet. However, if the mass is too viscous it will not be capable of extrusion at reasonable rates. The extruded mass of the bar soap compositions may be formed by cutting the extrudate into a final form of a bar soap having defined geometry. The extruded mass may be further optionally formed into a formed bar soap, such as by stamping or compressing a cut mass of the bar soap composition into a formed three-dimensional shape having a defined geometry.

By such a process, bar soaps of the invention may be made. Such extruded bar soaps have physical-chemical properties and an internal structure which are different from soaps that are made by a melt-cast process wherein a bar composition is first melted and liquefied in order to form a liquid phase which is then poured into moulds to solidify by quiescent cooling, after which the cooled "cast" bars may be removed and used.

Preferably the bar soaps formed form the bar soap compositions are rigid, self-supporting articles having a hardness as measured using a Humboldt Model H-1240 electric Penetrometer with a digital automatic timer of at least about 1.7 mm, but more preferably (and in order of increasing preference) exhibit a hardness of at least: 2, 2.25, 2.5, 2.75, 3, 3.25, 3.5, 3.75, 4, 4.25, and 4.5 mm of needle penetration, preferably as measured on a single bar soap sample. A single reading, or an average of a plurality of needle penetration readings (e.g., 2, 3, 5 or more readings), may be used in this evaluation. Again, unexpectedly the bar soap compositions of the present invention are found to be sufficiently durable for use in forming bar soaps therefrom by conventional processes, even though preferred bar soaps comprise in their soap constituent a high weight percentage of $C_6 - C_{16}$, and in particular C_{12} fatty acid soaps of potassium as such lower alkyl distributions in a soap constituent are frequently considered as being too soft for use in a product which is formed into a rigid, three-dimensional tablet or cake, viz., a bar soap, and which also exhibits a useful service life after repeated wettings with water. Surprisingly the bar soaps of the present invention are sufficiently hard and provide a satisfactory service life in their product format.

Subsequently the bar soaps may be packaged for sale as vendible products, e.g., overwrapped in a coated paper wrapper, packaged in a box, or even sold without any additional packaging.

The bar soaps are used in a conventional manner for personal washing of an mammalian body, e.g., human body and are advantageously used in personal washing, particularly of the epidermis, and hair. When used in a conventional washing process, typically the bar soap is wetted with water, and then contacted with one or more parts of the body, e.g., the epidermis, and hair. A quantity of the bar soap composition is thus eluted into the water and forms a washing composition which provides a useful cleaning and/or microbicidal benefit to the contacted parts of the body. The washing composition when entraining air, may form a lather which is also useful in providing a useful cleaning and/or microbicidal benefit to the contacted parts of the body. Thereafter the washing composition is typically washed or rinsed off the treated parts of the body, e.g., epidermis, hair, with an additional amount of water.

As the bar soaps of the invention are used in a conventional manner, they are used, as well as intended to be used, by contacting a bar soap with a quantity of water, which can be flowing water such as from a faucet, or can be a body (or aliquot) of water such as in a sink, or wash basin. Such contact between the soap bar and the water causes the dissolution or dispersion of the constituents of the bar soap into the water, viz., and "elution". This elution provides an effective antimicrobial benefit to a topical surface, particularly to the epidermis of a person or animal.

In particularly preferred embodiments, elutions formed from the partial dissolution of a bar soap composition or bar soap in water, which form aqueous dilutions of the bar soaps at concentrations of from 10 - 20 %w/v, (particularly preferably about 10%w/v) exhibit a pH in the range of at least about 9 more preferably a pH in the range of from about 9 – 10, more preferably from about 9.2 – 9.7, with particularly preferred pH values being identified with reference to one or more of the Examples. Desirably such elutions exhibit an antimicrobial benefit, particularly according to the testing protocol described with reference to the Examples.

In particularly preferred embodiments, aqueous compositions (elutions) of 16%wt. bar soap/water, preferably in deionized or distilled water, exhibit at least about a 2.0 log10 reduction of *E.coli, S. enterica, K. pneumonia, P. aeruginosa, E. aerogenes, E. faecalis, S. aureus* according to ASTM E2315 – 03 "Standard Guide for Assessment of Antimicrobial Activity Using a Time-Kill Procedure". More preferably such aqueous compositions (elutions) exhibit even higher levels of antimicrobial efficacy, preferably (and in order of increasing preference) at least about 2.5, 2.75, 3.0, 3.25, 3.5, 3.75, 4, 4.25, 4.5, 4.75, 5, 5.25, 5.5, 5.75, and even about 6 log10 reduction of according to ASTM E2315 – 03. Specific formulations, specific degrees of antimicrobial efficacy of tested aqueous elutions, according to ASTM E2315 – 03 are demonstrated amongst the Examples.

Examples

Foam Test

Material /Instruments

- Soap Bars
- Tap Water ± 25°C
- 1000ml Beakers
- 4 Graduated Cylinders
- Corn Oil
- Gerhardt machine
- Timer

In these examples the following exemplary formula was used (amounts in the final soap bar)

Soap Base (as noodles)	59	Surfactant
Starch	18	Filler
Sulfonic acids, sec. alkyl sulfonate	2	Surfactant
Sodium Isethionate	2	Surfactant

TCC	0.1	0.043
Sodium Carbonate / Sulphate	2	Structurant
Glycerine	1	Moisturiser
Fragrance	1.4	Fragrance
Titania	0.2	Opacifier
Dye	0.0250	Dye
Water	To 100	

Method of execution

1- Weigh ± 5 grams of bar soap and put it in the beaker

2- Add 1000 ml of tap water 25 °C

3- Stir until complete dissolution

4- Put 100ml of solution into the Gerhardt's cylinders

5- Stir for 2 min at speed 3

6- Register the level of foam

7- Add 4,5 ml of Corn oil in each cylinder

8- Stir for 2 min at speed 3

9- Register the final level of foam (the higher level the better the foaming properties)

Four formulations were tested. Two commercially available soap bars (Vanish and Sufi) and a formulation in accordance with the invention.

The results were as follows:-

Before the addition of the corn oil all showed a foam height in excess of 300ml. After the addition of the corn oil the foam heights were as follows.

Vanish: Foam height 225ml

Sufi: Foam height 300ml

Invention: Foam height 100-150ml

This shows how the In other words the formulation of the invention foams excellently when in use but said foam diminishes quickly allowing effective rinsing with low water usage.

Microbiology Test

EQUIPMENT LIST

Revolution shaker
Glass beads (3 - 4 mm) Transfer loops
Water bath capable of maintaining 50- 55 °C
Water bath capable of maintaining 37 ±I °C (shaking and /or circulating preferred)
Calibrated timer or stopwatch
Pipettes
Incubator capable of maintaining appropriate growth temperature for organism being tested
Test tubes
Vortex mixer
Refrigerator, capable of being controlled at 2-8°C Fluke K/J thermometer
Petri dishes Autoclave pH meter
Equipment to measure optical density/ transmittance (e.g. Biolog or spectrophotometer)
50mL Polypropylene Conical Tube Pipet Tips (100- 1000 microliter) Centrifuge

REAGENTS *I* MEDIA REQUIREMENTS

- Sterile purified water.
- Tryptic sodium chloride (TSC) solution, ingredients and directions listed below.

Tryptone, pancreatic digest of casein NaCl

Purified water	1 g
Mix and heat to dissolve.	8.5 g
	1 L

Tryptic soy agar (TSA). Agar containing extra neutralizers such as TSA+ (TSA with Lecithin and Polysorbate 80) and TSA-EXTRA can substitute TSA when test samples that are difficult to neutralize are tested . TSA and TSA+ dehydrated media can be purchased from a manufacturer. TSA- EXTRA ingredients and directions are listed below.

TSA-EXTRA TSA Lecithin

Tween 80	40.0 g
Purified water	7.0 g
	5.0 g
	1 L

Swirl as samples are aliquoted to ensure consistent distribution of ingredients. Heat to dissolve with frequent agitation , boil 1 minute.

Tween 80 can be heated separately first and allowed to cool for 3-5 minutes before being added to other components.

Neutralization liquid can be any validated mixture such as Dettol Neutralizer or *DIE* Broth. Dettol Neutralizer ingredients & directions are listed below. *DIE* Broth dehydrated media can be purchased from a manufacturer.

Dettol Neutralizer:

Tween 80	100 mL Lecithin 30.0 g Sodium
Thiosulfate Granular- Na2 S203	5.0 g
L-Histidine	1 .0 g
Phosphate Buffer	10 mL (see Phosphate Buffer below)
Purified water	900 mL

Tween 80 can be heated separately first and allowed to cool for 3-5 minutes before being added to other components.
Heat to dissolve with frequent agitation.
Transfer to end vessels and swirl to ensure consistent distribution of ingredients. Autoclave sterilize- afterwards, swirl thoroughly to ensure ingredients are dissolved. Equivalent dilutions may be made.

Phosphate Buffer:

Potassium Phosphate Monobasic Crystal, KH2P04	34.0 g
Purified water	1 000 mL

- EN Hard Water (prepared based on European Normal recommendations) for test sample dilution
(300ppm CaC03).

Solution A:

Dissolve 19.84 g of anhydrous MgCl2 and 46.24g of anhydrous CaCl2 in sterile purified water, and dilute to 1 litre (for example, in a volumetric flask or by weight).

These values can be adjusted for a different final volume (e.g. 1.98g MgC12 and 4.62g CaCb diluted up to l 00 mL in sterile purified water).

Solution B:

Dissolve 35.02 g of NaHC03 in purified water and dilute to 1 litre in a volumetric flask. These values can be adjusted for a different final volume.

EN Hard Water

To ~500 mL of sterile purified water add 6 mL of Solution A and 8 mL of Solution B, then dilute to 1 litre with sterile purified water using a volumetric flask. The pH of this solution should be between 6.8 and 7.2 at 25 °C. Where necessary, the pH of the Hard Water should be adjusted using 1M HCI or 1M NaOH.

Sterilize the final solution by passing through a membrane filter with an effective pore size of0.45 11m or 0.2 Jlm.

Solutions A & B can be stored at 2 °C to 8 °C for up to one month.

The final solution of Hard Water can be stored at 2 °C to 8 °C for up to one month.

TEST ORGANISMS

The following two organisms are tested unless otherwise stated:

Staphylococcus aureus	ATCC 6538
Escherichia coli	ATCC I 0536

Inoculate a TSA slant from frozen bead and incubate. Following incubation, transfer the culture to a 2"d TSA slant and incubate. A 3rd slant may be prepared and incubated. Use the 2"d or 3rd slant transfer in testing. Subcultures are prepared on TSA slants and incubated for I8 - 24 hours at 36 ± I 0 C. Refer to SOP No. *MN* 4/li for stock culture preparation. Alternatively, a second or third generation subculture from a culture purchased from a certified supplier can be used.

Other organisms can be tested when desired, and can be chosen from the following list when desired:

Corynebacterium xerosis	ATCC 373
Salmonella enterica	ATCC 10708
Staphylococcus epidermidis	ATCC 12228
Streptococcus pyogenes	ATCC 12384
Candida albicans	ATCC 10231
Candida tropicalis	ATCC 10610
Trichophyton mentagrohpytes	ATCC 9533

The appropriate growth medium and temperature should be used for each organism, for example Potato Dextrose Agar (PDA) at 30 ± 1°C for fungi. The yeast working culture must be a second or third generation subculture on the appropriate growth medium initiated from frozen beads and incubated at 30
± 1 °C for 24- 48 hours. The mould working culture is a first generation subculture on the appropriate
growth medium initiated from frozen beads and incubated at 30 ± I °C for 7- 9 days or until sufficient growth is observed.

PREPARATION OF TEST CULTURE Bacteria *I* Yeast:
Remove at least 2 loopfuls of the working slant and suspend the cells in approximately 10 mL of TSC and 5 g of glass beads and rotate at a rate of 150 revolutions per minute for at least three minutes.

Pipette a portion of the suspended cells and add to an appropriate volume of TSC (usually 9 mL or more depending on the amount of samples being tested). Adjust to give 1.5 - 5.0 x 1o8 CFU mL-I for *S. aureus* 6538 and *E. coli* 10536. A lower level of organism is acceptable for other bacteria as long as the range is above 1.0 x 10 7 CFU mL-1. For yeast the count should be above 1.0 x I 07 CFU mL-I as well. This level can be adjusted using a biolog transmittance kit, spectrophotometer, McFarland standard, or any other valid method of enumerating microorganisms.

A typical absorbance range of 45 to 50 (on the biolog) is used for bacteria, and 5 to IO for yeast. This becomes the test culture once adjusted to the correct level for each organism.

Mould:
Suspend the cells from the agar slant in saline by using a sterile loop and carefully detach the condiospores from the culture surface. Filter the spore suspension thru sterile glass wool. Transfer the suspension to a sterile conical flask (50 mL- 250 mL) containing 5g of glass beads (2mm-3mm). Rotate on a revolution rate of 150 min-1 for at least one minute.

Examine under the microscope immediately to show the absence of mycelial fragments and spore germination. If germinated spores are present the suspension shall be discarded . If mycelia are present the cells need to be washed (refer to the method for the centrifugation procedure - SOP No. *MN* 4/11). If necessary adjust the number of spores in the suspension to 1.5 -5.0 x107 CPU rnL- 1 using the diluent (saline).

This becomes the test culture and shall not be stored for more than 2 days at 2 - 8 °C.

PREPARATION OF TEST SAMPLE

Liquid Handwashes, Shampoos, Leave-On Samples, and Wipes:
All wash-off liquid samples should be tested at a final in-test dilution of 50%v/v unless otherwise stated. This dilution is achieved when the 1 mL of test culture is added, i.e. 5 mL sample, 4 mL Hard Water and 1 mL test culture.

For leave-on samples and wipes (expressed liquid), no dilution is necessary and testing should proceed with a final in-test dilution of 90% v/v. The procedure to extract the liquid (expressed liquid) from the wipes is explained below.

Expressed Liquid - Insert five pipet tips (100 - 1000 microliter) into a 50m L Polypropylene Conical Tube and aseptically place as many wipes as possible into the upper part of the tube. Place closure cap on tube and centrifuge for 3 minutes (3000g, 24°C). The expressed liquid will collect at the bottom of the tube. Discard wipes and aseptically remove the expressed liquid.

Bar Soaps (hours prior to test):

Bar soaps should be tested as a 16% w/v solution. The soap suspension is initially prepared at a concentration 1.11 times this required level, to allow for dilution of the sample in test by the addition of 1 mL inoculum to 9 mL sample suspension.

1. At least half of a bar soap should be grated at one time to ensure consistency of the grated product. Grate the soap bar using a clean food processor or other suitable instrument (e.g. a cheese grater) to produce small chips or powder of soap. Transfer 17.76g of these chips or powder to a suitable sterile glass bottle. Once a bar of soap has been grated, it must be used within 36 hours.

2. Add 82.24 mL of EN Hard Water to the soap chips or powder to suspend the soap on the day of testing. The EN Hard Water can be placed at room temperature for 24 hours prior to addition to the soap, to allow for fasterdissolving.

3. Leave the solution at 50 °C to 55 °C in the water bath with occasional swirling until the soap has dissolved completely. This usually takes 1-2 hours. The temperature of the water bath should be monitored using a calibrated thermometer (e.g. Fluke).

TEMPERATURE

Test solutions must maintain a temperature of 37 ± 1 °C in a water bath (shaking or circulating preferred) unless otherwise stated. Monitor using a calibrated thermometer (e.g. Fluke).

TEST SUBSTANCE EQUILIBRATION EVALUATION

Use the applicable procedure to determine the amount of time required for test sample equilibration. One control tube for each product will be evaluated . Ensure the sample mixture is below the waterline of the 37± 1 °C water bath.

Liquid Handwashes and Shampoos Control Tube:
Bring the Hard Water up to room temperature to ensure consistency. Add 4 ml of the hard water to 5 mL of a representative sample and place in the test water bath that is at 37 ± 1°C. Immediately place a calibrated thermometer in the representative sample and begin a timer. Record the time it takes for the sample to reach the test temperature range 37 ± 1 °C. Once complete, discard the sample. For the test procedure, use the time recorded to warm up the sample as an estimate for equilibration.

Liquid Leave-On Samples and Wipes (Expressed Liquid) Control Tube:
Place 9 mL of the representative sample in the test water bath that is at 37 ± 1 °C. Immediately place a calibrated thermometer in the representative sample and begin a timer. Record the time it takes for the sample to reach the test temperature range 37 ± 1 °C. Once complete, discard the sample. For the test procedure, use the time recorded to warm up the sample as an estimate for equilibration.

Bar Soaps Control Tube:
Once the soap has dissolved in the water bath (50- 55 °C), take a representative aliquot of sample (9 mL) and place in the test water bath that is at 37 ± 1 °C. Immediately place a calibrated thermometer in the representative sample and begin a timer. Record the time it takes for the sample to reach the test temperature range 37 ± 1 °C. Once complete, discard the sample. For the test procedure, use the time recorded to cool down the sample as an estimate for equilibration.

CONTACT TIME

Samples should be tested using a 1 minute contact time unless otherwise stated .

TEST PROCEDURE

To be performed at 37 ± 1 °C.

1. In a sterile 20 x 150 mm culture tube (or otherwise appropriate sterile container, e.g. a McCartney bottle) add 4 mL Hard Water to 5 mL of a liquid handwash sample. For liquid leave-on

samples and wipes (as an expressed liquid), dispense 9 mL of the sample in a culture tube. For bar soaps, dispense
9 mL of the dissolved soap into a culture tube. Dissolved bar soaps should be kept at 50- 55 °C until
the next step is performed.

2. Allow sample mixture to equilibrate in the water bath for the determined equilibration time. Ensure the sample mixture is below the waterline of the 37 ± 1 °C water bath. Also include a tube containing 8mL Neutralizer and 1 mL sterile purified water for each sample tested and allow this to equilibrate at the testing temperature.

3. Add 1 mL of test culture (which has equilibrated at the test temperature) to the tube containing the sample and Hard Water, and vortex for 5 seconds. Leave for the 1 minute± 5 second contact time.

4. After the contact time, vortex and remove 1 mL of the test mixture (for viscous samples such as shampoo or bar soap, a syringe or positive displacement pipette may be used) and add to a tube containing 8 mL neutralizer and 1 mL water, which has equilibrated at the test temperature. The
resultant inactivated mixture is a 10-1 dilution of the test reaction mixture(*).

5. Allow a 5 minute neutralization period. Perform serial dilutions of 1 in 9 mL TSC (or 0.1 mL in 9.9 mL TSC) to 10-5 unless otherwise stated and plate 1ml of the appropriate aliquots of dilutions to enumerate the surviving organisms. Plating should be done in duplicate unless otherwise stated. Pour plates with molten the appropriate growth medium, which has been maintained at a temperature of 45
-50 °C.

6. Repeat steps 1 to 5 using a maximum of 3 samples per 1 minute contact time run and thus a 20 second interval between each.

7. Incubate at 36 ± 1 °C for bacteria, 30 ± 1 °C for yeast or mould, or the appropriate growth

temperature for 2- 3 days or until sufficient growth is observed and count colonies or spores.

8. Perform two replicates of the procedure, or as per the sponsor.

* 1 mL in 9 mL is the standard neutralization dilution. Where this is not applicable due to insufficient neutralization a 0.1 mL in 9.9 mL neutralization can be used.

COUNTING OF TEST CULTURE

1. Dilute each organism to 10-7 in TSC using appropriate dilutions after performing the test procedure.
If a test procedure runs over a long period of time, separate dilutions should be performed to represent different p01tions of the day during which testing was performed. For example, if a test is performed through a full day, there should be one dilution done in the morning and one in the afternoon.

2. Plate the 10-5 , 10-6 and 10-7 dilutions in duplicate and pour with TSA.

3. Incubate at 36 ± 1 °C for bacteria, 30 ± 1 °C for yeast or mould, or the appropriate growth temperature for 2- 3 days or until sufficient growth is observed and count colonies or spores.

VALIDATION OF NEUTRALIZATION

To be conducted for each test sample/organism combination. Ensure all tubes have been equilibrated as per the Test Procedure section.

1. Neutralization must be validated at least once for each new test sample *I* organism combination tested.

2. Prior to the test procedure dilute the test culture using TSC to give a cell concentration of 103CFU
mL- 1 by performing five ten-fold dilutions in TSC.

3. Allow the test samples and 8mL Neutralizer tube to equilibrate in the water bath for the determined equilibration time.

4. Add 1 mL ofTSC to each sample solution, vortex and leave for 1 minute.

5. Remove 1 mL of the test mixture to 8 mL neutralizer, vortex and leave for 5 minutes.

6. Add 1 mL of the test culture (as per 2) to the neutralized mixture and leave for 30 minutes.

7. Plate the neat and -1 dilutions of the neutralized mixture in duplicate (1 mL and 0.1 mL in duplicate)
and pour plates with molten TSA.

8. Repeat steps 3 - 7, replacing the test sample with 9mL of EN Hard Water. This will serve as the control for neutralization validation .

satisfactory

9. Incubate at 36 ± 1 °C for bacteria, 30 ± 1 °C for yeast or mould, or the appropriate growth temperature for 2- 3 days or until sufficient growth is observed and count colonies or spores.

10. Neutralization is effective if the number of organisms in the inactivated test sample mixture is at least within 0.5 log of that recovered from the control.

CALCULATION AND EXPRESSION OF THE RESULTS

Where possible, plates containing between 15 and 300 CFU/mL should be used to derive counts.

The Weighted Mean
The weighted mean is the average of choice, and is used when two dilutions have a number of colonies
(counts) in the range of 15-300 CFU/mL.

Use counts from two successive dilutions as follows:

Where double plates are used, add up all four plates and divide by 2.2 (all plated must be between 15 and 300). Multiply this number by the reciprocal of the highest dilution as shown below:
Example (double plating)
1o-l counts: 280, 290
1o-2 counts: 40, 52
280 + 290 + 40 + 52 = 662
662/2.2 = 301
301 x lO(reciprocalofl0-1)=3.01 x 103

Where single plates are used, add both plates (both plates must be between 15 and 300) and divide by 1.1. Multiply this number by the reciprocal of the highest dilution as shown below:
Example (single plating)
1 0"1 counts: 280
10"2 counts: 40
280 + 40 = 320
320/1.1 = 291
291 x 10 (reciprocal of 10"1) = 2.91 x 103

Weighted means can be used in combination. For example, if one of the 10·1 plate counts is within range and two of the 10·2 plate counts are within range, these three counts can be added and the total divided by 1.2. This value can be multiplied by the reciprocal of the highest dilution.

Dilution Reciprocal
Highest dilution= 10·3 = 0.001
Dilution factor= 0.001
Reciprocal= 0.001·1 = 1/0.001 = 1000

The Arithmetic Mean
The arithmetic mean is used when only one dilution has colonies in the range 15 - 300 CFU/mL and duplicate plating is used. Divide the mean of the two numbers by two.
Like the weighted mean, multiply the dilution reciprocal as shown above.

Example (double plating)

10"1 counts: 280, 290

280 + 290 = 570

570/2 = 285

285 X 10 (reciprocal Of} 0"1) = 2.85 X 103

Handling of data with no survivors or uncountable plates

When no survivors are recovered, for the purpose of calculation consider the number of colonies recovered to be <15, multiplied by the reciprocal of the dilution factor.

Example:

10·1 counts: 0, 0

10"2 counts: 0, 0

<15 x 10 (reciprocal of 10"1) = <1.5 x 102

The log value of this is <2.18. As no actual organisms were recovered, insert a ">" symbol before the calculated Microbial Effect (ME) value.

Handling of data with one countable plate in single plating

When one plate has colonies in the range of 15- 300 CFU/mL, that value will multiplied by the reciprocal of the dilution from which it was plated. No averages will be taken.

Example (single plating)

10·1 count: 310

10·2 count: 35

10"3 count: 4

35 x 100 (reciprocal of 10"2) = 3.5 x 103

When all plates are uncountable (TNTC), consider the mean number of colonies in the greatest dilution to be

>300x in the reciprocal dilution factor.

Example:

10·1 counts: 329, 348

>300 x 10 (reciprocal ofl0-1) = >3 x 103

The log value of this is >3.48. As uncountable organisms were recovered, insert a "<" symbol before the calculated ME value.

Calculation of microbiocidal effect (log reduction)

The microbiocidal effect (ME) due to the action of the test sample over the test contact time at the temperature at which the test was performed is expressed by the formula:

ME= Log Nc- Log Nd

Where: Nc = number of CFU/mL of the in test culture count (1:10 of the initial suspension population control result)

Nd =number of the CFU/mL of the sample count

Calculate to 2 decimal places.

The results were as follows:

Sample Information	***S. aureus*** **Log_{10} Reduction 60 sec**	***E. coli*** **Log_{10} Reduction 60 sec**
Multi-Purpose Bar According to the invention	1.49, 1.50, 1.49 **(1.50)**	1.48, 1.40, 1.61 **(1.50)**
Multi-Purpose Bar According to the invention	1.85, 1.72, 1.90 **(1.82)**	1.48, 1.48, 1.26 **(1.41)**

Fragrance Microbiology Test

Fragrance Microbiology Test

The fragrance was tested similarly according to ASTM E2315 – 03 "Standard Guide for Assessment of Antimicrobial Activity Using a Time-Kill Procedure". The test Aromahygiene® fragrances, Aromahygiene® is a registered trademark of Takasago International Corporation (USA), were diluted in ethanol and added to deionized water at various concentrations. Total amount of ethanol in the test solution was no more than 2% which did not affect activity. Microorganisms (*Escherichia coli* ATCC 10536, *Staphylococcus epidermidis* ATCC 12228) were grown overnight in an enriched nutrient broth and sampled to determine the bacterial number. They were added to the test solutions, mixed using a vortex mixer and incubated at room temperature. Samples were removed at various fixed time intervals and diluted into D/E Neutralization broth (Difco, Detroit, MI, USA). The treated samples were then spiral plated onto solid media and incubated at 37°C for 24 hours. Colony-forming units were then counted and normalized as log cfu/ml. Activity was calculated as the difference between initial counts versus final counts at that time interval.

Table 1

	E. coli Log_{10} Reduction 60 seconds
Citrus Clean @ 0.2%	1.70
Citrus Clean @ 1%	3.68
Citrus Fresh @ 0.2%	1.89
Citrus Fresh @ 1%	3.57

Table 2

	E. coli Log_{10} Reduction 10 minutes	*E. coli* Log_{10} Reduction 30 minutes	*E. coli* Log_{10} Reduction 1 hour
Citrus Clean @ 0.2%	2.21	2.53	3.24
Citrus Clean @ 1%	>5	>5	>5
Citrus Fresh @ 0.2%	3.91	>5	>5
Citrus Fresh @ 1%	>5	>5	>5

Table 3

	S. epidermidis Log_{10} Reduction 10 minutes	*S. epidermidis* Log_{10} Reduction 30 minutes	*S. epidermidis* Log_{10} Reduction 4 hours
Citrus Clean @ 0.2%	1.25	1.51	1.54
Citrus Clean @ 1%	1.68	2.04	2.17
Citrus Fresh @ 0.2%	0.83	1.32	1.40
Citrus Fresh @ 1%	2.19	2.58	4.00

Table 4

Citrus Fresh RTA-005280 0.2%

	10min	30min	1 hour	4 hours
E. coli	99.99%	>99.99%	>99.99%	>99.99%
S. aureus	68.38%	84.15%	82.82%	97.57%
C. minutissimum	94.93%	99.93%	99.99%	>99.99%
S. epidermidis	94.96%	94.04%	96.20%	94.77%

Table 5

E. coli

	1min	3min
Citrus Clean RTA-005279 0.2%	97.98%	99.65%
Citrus Clean RTA-005279 1%	99.98%	>99.99%

Table 6

E. coli

	1min	3min
Citrus Fresh RTA-005280 0.2%	98.70%	99.63%
Citrus Fresh RTA-005280 1%	99.97%	>99.99%

Malodour Testing Protocol

- Malodor
  - Kitchen Malodor (proprietary composition from Takasago International Corporation (USA))
  - Sweat Malodor (proprietary composition from Takasago International Corporation (USA))
- Protocol Using Smelling Jar
  - Place 7.5cm filter paper in disposable plastic petri dish
  - An aliquot of warm soap solution is placed onto the filter paper. Malodor is applied on top.
  - Place petri dish in 3-digit random coded glass jar
  - Cap the jar and allow to equilibrate for 30 minutes prior to evaluation
  - The Malodor reference jar (no fragrance) will be presented to the panel before coded sample jars

Jar Specifications:
2 litre wide-mouth glass jar
Plastic lid to fit with 5/8" hole drilled at 6 o'clock and ¼" hole drilled at 12 o'clock
Disposable plastic pipette to fit in the ¼" hole(23 ml capacity, 7.3 ml bulb draw), cork to fit 5/8" hole

Malodor	Malodor Reduction (%)	Significance* (Pass/Fail)
Sweat	57% - CITRUS FRESH 59% - CITRUS CLEAN	Pass
Kitchen	74% - CITRUS FRESH 66% - CITRUS CLEAN	Pass

Mildness Test

Background

In a broad sense, the more a product or a surfactant affects the natural state of skin by increasing skin redness, dryness, and roughness, the harsher it is (Lukacovic, Micheal F et al, Forearm wash test to evaluate the clinical mildness of cleansing product, j. Soc. Cosmet. Chem., 39, 355-366 (November/December 1988)). Therefore, a formulation which does not adversely affect the above parameters is said to be milder on a relative scale.

Mildness can be measured using multiple methods each with its own merits.

Use of Zein to measure mildness of personal care products is widely reported in literature (Xia, Jiding, Protein-Based Surfactants: Synthesis: Physicochemical Properties, and Applications, Page 231, CRC Press, June 2001; Mengtao He, Bar composition comprising nonionic polymeric surfacing as mildness enhancement agents, Patent US 5795852 A, Lever Brothers Company, Division Of Conopco, Inc., 1998).

Stratum corneum (SC) has a brick and mortar like structure of dead cells where the bricks correspond to keratin and the mortar to lipids. When a surfactant interacts with the skin, it dissolves the keratin in the SC thus damaging the outer protective layer of the skin (Ananthapadmanabhan KP, Cleansing without compromise: the impact of cleansers on the skin barrier and the technology of mild cleansing, 2004). When there is excessive exfoliation, the skin starts losing more moisture than normal due to the damage in the protective upper layer of SC. When this level reaches below 10 % water content, the skin becomes dry, scaly and itchy (see Lukacovic above). So, when a surfactant damages the layers of SC, it increases water loss leading to dry skin. This is correlated with harshness. The harshness of anionic surfactants with compact head groups has been suggested to be due to their ability to bind to SC, especially the SC proteins. The exact mechanism has also been identified where the surfactant starts interacting with the proteins at their active sites. As the surfactant concentration increases, more and more molecules get into the structure of protein and start binding. This causes an increase in the steric hindrances and electrostatic repulsion between the two types of molecules. These forces eventually break apart the quaternary and tertiary structure of protein which in turn eases its solubilization (Ananthapadmanabhan, Kavssery. P., Role of Surfactant Micelle Charge in Protein

Denaturation and Surfactant-Induced Skin Irritation, chapter 9: Surfactants in Personal

Care Products and Decorative Cosmetics, Third Edition, CRC Press 2006, Pages 177–187). Figure 2 shows how the surfactant interacts at the active sites of the protein resulting in denaturation and unfolding of it.

Since keratin is insoluble in water, the amount of protein solublized in the presence of a surfactant solution can be directly correlated with the harshness of that surfactant - more the dissolution of keratin from the skin, the harsher is the surfactant. However, keratin is not readily available for experimentation and zein is a cheap and suitable alternative.

Method Development

Materials Required

- 10 gms of sample to be tested
- Protein Zein (W555025 from Sigma Aldrich)
- SLES-70% surfactant (Galaxy surfactants, India)
- Ethanol 99.9% (Changshu Yangyuan Chemicals, China)
- Syringes with volume atleast 3 ml
- 0.22 µm syringe filter (nylon)

Methodology

A 1 % solution of the formulation was made in distilled water. If it was not possible to operate at 1 % for any reason, a lower concentration was chosen for experimentation. Then, a 100 ml of this solution was taken for evaluating Zein solubility. This was done in triplicates. The rest was used as a blank for the experiment.The solution was kept under stirring at 250 rpm. Two grams of Zein was added to the solution and the stopwatch was set off simultaneously. The mixture was left on stirring for 10 minutes after which the solution was left undisturbed for one minute. After the un- dissolved Zein settled, a sample was extracted from the solution avoiding the top and the bottom layers using a syringe. The extract was then filtered using a 0.22 µm syringe filter into a vial. It is worth while noting the solutions though hazy at this point, become clear with further dilutions. The robustness of this technique was established by checking for a linear signal at various concentrations of zein. The extract was then diluted to a factor of
25 - 50 or whatever suitable otherwise. Absorbance readings were taken using a Perkin Elmer Lambda 25 UV/VIS Spectrophotometer at 277-278 nm). The experiment was always conducted

with SLES (1 %) solution as control. Solutions of the respective products at similar concentrations were used as blanks.

Results and Discussion

Calibration Curve: Solutions with known concentrations of Zein were prepared in a 70% ethanol solution to construct a calibration curve. The data obtained is tabulated inTable 2 and plotted in Figure 3.

y (absorbance) = 0.0009 x - 0.109

where x is the concentration in ppm. The R^2 value is 0.9999.

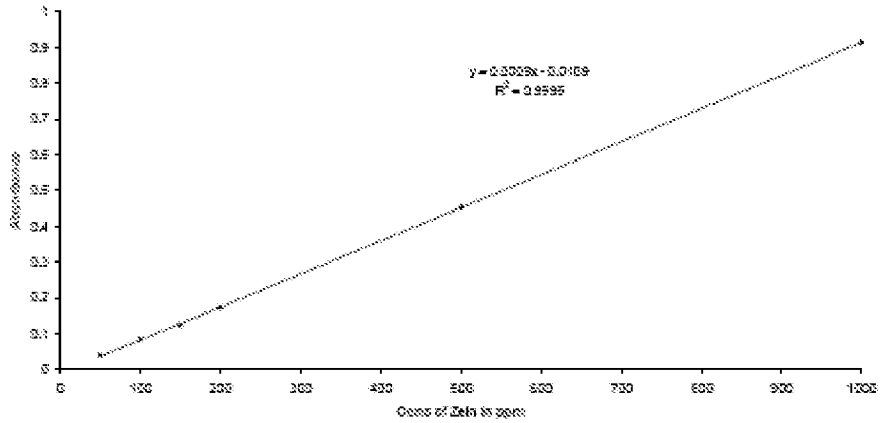


Figure 3: Calibration Curve for Zein in ethanol

Validation of modifications in standard zein solubilization test

To ensure that the method currently employed (dissolving and measuring zein in a surfactant solution at 278 nm) is robust enough, linearity of the signal in a surfactant solution was checked.

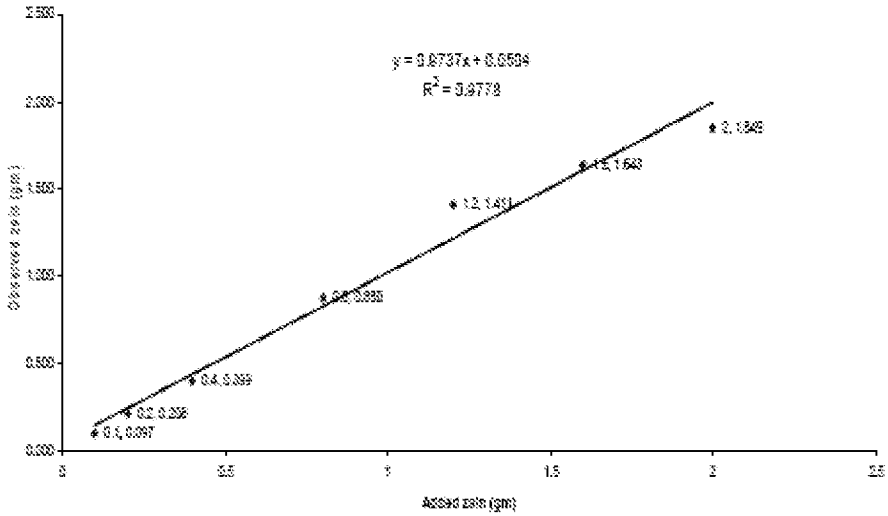


Figure 4: Linearity of response for zein solubilization in a surfactant solution

The dissolution time for Zein in any given surfactant as reported in literature varies from as little as 10 minutes to 24 hours. However, in order to make the method we employ in house more robust, we checked the kinetics of Zein dissolution in SLES. The results show that the SLES solution solubilizes all the Zein in approximately 10 minutes. Therefore, 10 minutes was decided as the time point of sample extraction. The results of this part are shown in Table 4 and Figure 5.

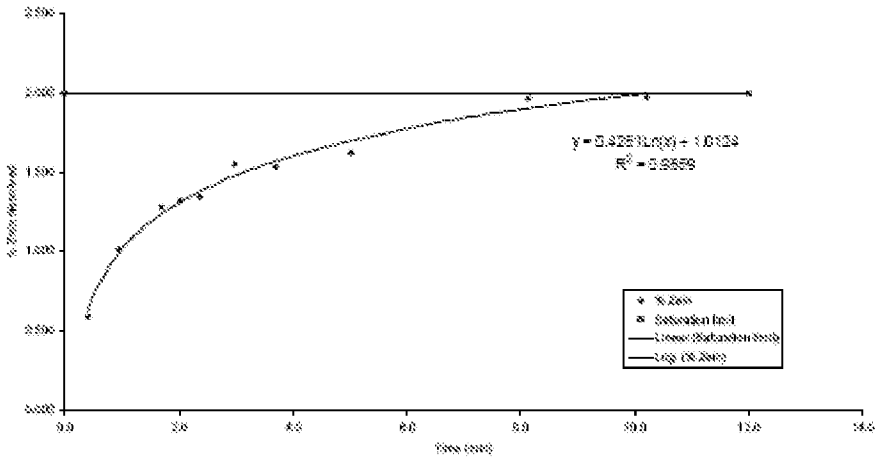


Figure 5: Kinetics of dissolution of zein in SLES

Formulation A (in accordance with the invention) and Formulation B (comparative formulation) bar soaps (at 0.5 % w/v concentration in distilled water) were tested using this method with SLES (1 %) as the positive control. The final results of the experiment are mentioned in Table 5 and Figure 6.

It is clear from the data shown (figure 6 and table 5) that the Formulation B soap dissolves more than twice the amount of zein as the Formulation A bar soap. Hence, it is safe to consider that the Formulation A bar soap is at least 2 times milder than the existing (Formulation B) soap formulation.

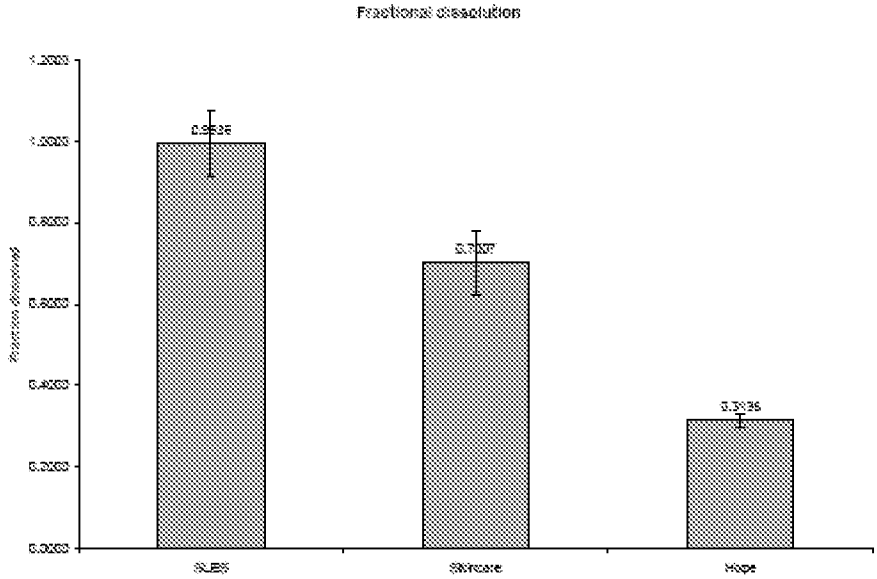


Figure 6: Zein dissolution by Dettol Skincare and Hope bar soaps

Considerations

The effect of isoelectric point and pH on the experiment was also taken into consideration. The solubility profile vs. pH of zein follows the same trend as keratin. Though insoluble in water, both have significant solubility in alkaline pH conditions which cannot be neglected.

- Option 1: Compare the solubility of zein by artificially controlling the pH for all samples (nullify the component of solubility that comes from the change in pH of the solution after adding surfactants)

- Option 2: Let the pH be what it tends to be naturally in the surfactant solution for comparison. This will be a function of the formulation used.

To mimic real life conditions, all formulations were tested without adjusting for pH. This, we believe, is closer to the real consumer experience.

Appendix

Table 2: Data points for calibration Curve

S. No.	Wavelength (nm)	Conc. (ppm)	Absorbance
1	278	50	0.0385
2	278	100	0.0827
3	278	150	0.1226
4	278	200	0.1727
5	278	500	0.4544
6	278	1000	0.9130

Table 3: Linearity validation data

S. No.	% Zein added	Absorbance	Conc in cuvette (ppm)	Dilution factor	Actual conc (g/ml)	%conc
1	0.1	0.0767	97.31	10	0.001	0.097
2	0.2	0.0639	83.09	25	0.002	0.208
3	0.4	0.1328	159.70	25	0.004	0.399
4	0.8	0.3058	351.89	25	0.009	0.880
5	1.2	0.2431	282.22	50	0.014	1.411
6	1.6	0.2848	328.53	50	0.016	1.643
7	2	0.3219	369.81	50	0.018	1.849

Table 4: Kinetics of dissolution of zein in SLES

S.N	Time (min)	Absorbance	Conc in cuvette	Dilution factor	Actual conc	%conc
1	0.4	0.095	117.67	50	0.006	0.588
2	1.0	0.172	203.22	50	0.010	1.016
3	1.7	0.220	256.56	50	0.013	1.283
4	2.0	0.227	264.33	50	0.013	1.322
5	2.4	0.231	268.78	50	0.013	1.344
6	3.0	0.268	309.89	50	0.015	1.549
7	3.7	0.265	306.56	50	0.015	1.533
8	5.0	0.281	324.33	50	0.016	1.622
9	8.1	0.343	393.22	50	0.020	1.966
10	10.2	0.345	395.44	50	0.020	1.977

Table 5: Zein dissolution by Formulation B and Formulation A Bar soaps

SLES								
S. No.	SET	% Zein added	Absorbance	Conc (ppm)	Dilution factor	Actual conc	%conc	Relative ZN
1	A	2	0.6427	726.22	25	0.0182	1.816	0.908
2		2	0.6167	697.33	25	0.0174	1.743	0.872
3		2	0.7417	836.22	25	0.0209	2.091	1.045
4	B	2	0.7570	853.22	25	0.0213	2.133	1.067
5		2	0.7327	826.22	25	0.0207	2.066	1.033
6		2	0.736	829.89	25	0.0207	2.075	1.037
		A	Hope final	8-Dec	SD	Avg ZN		
		B	SC final	12-Dec	0.08206	0.9936		
Formulation A								
S. No.	SET	% Zein added	Absorbance	Conc in cuvette	Dilution factor	Actual conc	%conc	Relative ZN
1	N	2	0.2082	243.44	25	0.00609	0.609	0.30431
2		2	0.2274	264.78	25	0.00662	0.662	0.33097
3		2	0.2091	244.44	25	0.00611	0.611	0.30556
		N	final	8-Dec	SD	Avg ZN		
					0.01505	0.3136		
Formulation B								
S. No.	SET	% Zein added	Absorbance	Conc in cuvette	Dilution factor	Actual conc	%conc	Relative ZN
1	Y	2	0.5179	587.56	25	0.01469	1.469	0.73444
2	Z	2	0.5420	614.33	25	0.01536	1.536	0.76792
3		2	0.4110	468.78	25	0.01172	1.172	0.58597
4		2	0.5020	569.89	25	0.01425	1.425	0.71236
		Y	A final	8-Dec	SD	Avg ZN		
		Z	B final	12-Dec	0.07949	0.7002		

– Formulation A bar soap:

Item description	%w/w
Soap Base (as noodles)	59
Starch	18
Sodium isethionate	2
Sodium C14-17 alkyl sec sulfonate	2
Sodium Carbonate	1
Sodium Sulfate	1
TCC	0.1
Glycerine	1
Fragrance	1.4
Titanium dioxide	0.2
Water	To 100

5

– Formulation B bar soap:

Item description	%w/w
Soap Base (as noodles)	82.2298
Talc	9
Titanium dioxide	0.3
AOS POWDER	0.75
TCC	0.2
Lauryl Lactyl Lactate	0.35
Stearyl Heptanoate	0.2
Sucrose Cocoate	0.25
Glycerine	1.1
Silicone	0.5
Optical Brightener	0.02
Vitamin C	0.0001
Niacinamide	0.0001
Sodium Sulphate	0.6
Water	3.5
Fragrance	1
Total	100

BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY

INTRODUCTION

The Bovine Corneal Opacity and Permeability Assay (BCOP) was used to assess the potential ocular irritancy of the test articles to isolated bovine corneas. Bovine corneas, obtained as a by-product from freshly slaughtered animals, were mounted in special holders and exposed to the test articles. An *in vitro* score was determined for each test article based on the induction of opacity and permeability (to fluorescein) in the isolated bovine corneas.

The purpose of this study was to evaluate the potential ocular irritancy of the test articles, as measured by changes in opacity and permeability (to fluorescein) in isolated bovine corneas.

MATERIALS AND METHODS

Bovine Eyes

Bovine eyes were obtained from freshly slaughtered animals (J.W. TREUTH & SONS, Inc., Baltimore, MD). The eyes were excised and then placed in Hanks' Balanced Salt Solution, containing penicillin/streptomycin (HBSS), and transported to the laboratory on ice packs. Immediately upon receipt of the eyes into the laboratory, preparation of the corneas was initiated.

Preparation of Corneas

The eyes were grossly examined for damage and those exhibiting defects were discarded. The tissue surrounding the eyeball was carefully pulled away and the cornea was excised such that a 2 to 3 mm rim of sclera was present around the cornea. The isolated corneas were then stored in a petri dish containing HBSS until they were mounted in a corneal holder. The corneas were mounted in the holders with the endothelial side against the O-ring of the posterior chamber. The anterior chamber was then positioned on top of the cornea and the screws were tightened. Starting with the posterior chamber, the two chambers were then filled with Minimum Essential Medium (EMEM) without phenol red, containing 1% fetal bovine serum and 2 mM L-glutamine (Complete MEM (without phenol red)). Each corneal holder was uniquely identified with a number written in permanent marker, on both the anterior and posterior chambers. The corneal holders were incubated at 32 ± 1°C for a minimum of 1 hour.

Controls

The positive control used in this study was ethanol (Pharmco). The negative control used in this study was sterile, deionized water (Quality Biological).

Test Article Preparation

Each test article was administered to the test system as a 10% (w/v) (100 mg/mL) dilution in sterile,

deionized water. Each test article dilution was prepared by weighing the test article into a prelabeled conical tube. Sterile, deionized water was added until a 10% (w/v) dilution was achieved, the conical tube was vortexed for approximately 2 minutes, and then the conical tube was placed on a plate shaker for ~23 hours at room temperature. After shaking overnight, each dilution was centrifuged at 100 x g for 1 minute at room temperature to separate the foam bubbles from the dilution. Each dilution was dosed onto the corneas within 15 minutes of removal from the plate shaker. For the remainder of this report, each test article dilution is referred to as the test article.

Test Article pH Determination

The pH of each test article was determined using pH paper (EMD Millipore Corporation). Initially, each test article was added to 0-14 pH paper with 1.0 pH unit increments to approximate a narrow pH range. Next, each test article was added to 7.5-14 pH paper with 0.5 pH unit increments. The pH values obtained from the narrower range pH paper are presented in Table 1.

Bovine Corneal Opacity and Permeability Assay

After a minimum of 1 hour of incubation, the corneas were removed from the incubator. The medium was removed from both chambers and replaced with fresh Complete MEM (without phenol red). The initial opacity was determined for each cornea using an Electro Design OP-KIT opacitometer. The treatment of each cornea was identified with the test article number written in permanent marker on colored tape, affixed to each holder. The medium was then removed from the anterior chamber and replaced with the test article, positive control, or negative control.

Method for Testing Liquid or Surfactant Materials

The liquid test articles, formulation of the invention (as above) and Johnson's Baby Bar (comparator), were tested as 10% (w/v) dilutions in sterile, deionized water. An aliquot of 750 µL of the test article, positive control, or negative control was introduced into the anterior chamber while slightly rotating the holder to ensure uniform distribution over the cornea. Due to their viscous nature, the test articles were administered to the corneas by open chamber technique. In the open chamber technique, the glass window was removed from the anterior chamber immediately prior to treatment, and then the test articles were administered by direct addition onto the epithelial surface of the cornea using a positive displacement pipet. Each cornea was completely covered with test article. After dosing, the glass window was replaced on the anterior chamber. Three corneas were incubated in the presence of the positive control at 32 ± 1°C for 10 minutes. Three corneas were incubated in the presence of the negative control at 32 ± 1°C for 10 minutes. Five corneas were incubated in the presence of each test article at 32 ± 1°C for 10 minutes. After the 10-minute exposure time, the control or test article treatments were removed. The epithelial side of the corneas was washed at least three times with Complete MEM (containing phenol red) to ensure total removal of the controls. The corneas were then given a final rinse with Complete MEM (without phenol red). For the corneas treated with the test articles (open chamber technique), the glass window was removed from the anterior chamber and the test article was rinsed from the treated cornea and the anterior chamber with Complete MEM (with phenol red). Care was taken not to spray the corneas directly. The chamber windows were returned to the chambers when most or all of the test article had been removed. The rinsing process continued in the same manner as the

positive and negative control-treated corneas. The corneas were then given a final rinse with Complete MEM (without phenol red). The anterior chambers were refilled with fresh Complete MEM (without phenol red) and an opacity measurement was performed. The corneas were returned to the incubator for approximately 2 hours after which a final measure of opacity was obtained.

After the final opacity measurement was performed, the medium was removed from both chambers of the holder. The posterior chamber was filled with fresh Complete MEM (without phenol red) and 1 mL of a 4 mg/mL fluorescein solution was added to the anterior chamber. The corneas were then incubated in a horizontal position (anterior side up) for approximately 90 minutes at 32 ± 1°C. At the end of the 90-minute incubation period, the medium was removed from the posterior chambers and placed into tubes numbered with corresponding chamber numbers. Aliquots of 360 µL from the numbered tubes were placed into their designated wells on a 96-well plate. The optical density at 490 nm (OD_{490}) was determined using a Molecular Devices *V*max kinetic microplate reader. The plate reading was saved to a designated print file.

Presentation of Data

Opacity Measurement: The change in opacity for each cornea (including the negative control corneas) was calculated by subtracting the initial opacity reading from the final opacity reading. These values were then corrected by subtracting from each the average change in opacity observed for the negative control corneas. The mean opacity value of each treatment group was calculated by averaging the corrected opacity values of each cornea for that treatment condition.

Permeability Measurement: The mean OD_{490} value for the blank wells was calculated. The mean blank OD_{490} value was then subtracted from the raw OD_{490} value of each well (corrected OD_{490}). The final corrected OD_{490} values of the test articles and the positive control were then calculated by subtracting the average corrected OD_{490} value of the negative control corneas from the corrected OD_{490} value of each treated cornea:

Final Corrected OD_{490} = (raw OD_{490} – mean blank OD_{490}) – average corrected negative control OD_{490}

The mean OD_{490} value of each treatment group was calculated by averaging the final corrected OD_{490} values of the treated corneas for that treatment condition.

The following formula was used to determine the *in vitro* score:

In Vitro Score = Mean Opacity Value + (15 x Mean OD_{490} Value)

Criteria for Determination of a Valid Test

The BCOP assay was accepted when the positive control (ethanol) caused an *in vitro* score that fell within two standard deviations of the historical mean.

RESULTS AND DISCUSSION

Bovine Corneal Opacity and Permeability Assay

Table 1 summarizes the opacity, permeability, and *in vitro* score for the test articles and the positive control. Since the results of the positive control fell within two standard deviations of the historical mean (within a range of 39.1 to 63.7), the assay was considered valid.

A post-exposure (PE) opacity reading was performed immediately after rinsing and then a final opacity reading was performed after the 2 hour post-exposure incubation period. The PE reading was performed to capture immediate effects of the test article to the cornea (e.g. cloudiness of the cornea). Only the final opacity value was used to determine the change in opacity for data calculations (determination of In Vitro Irritation Score). Certain types of products (e.g. those which contain surfactants) may show immediate increases in opacity (as determined at the PE reading), and then result in a lower opacity value at the final opacity determination. One cornea (number 16) treated with the test article, the formula of the invetion, had a decrease of 5 opacity units from the PE opacity to final opacity determination; and all remaining corneas treated with this test article changed 1 to 3 opacity units from the PE reading to the final reading.. All of the corneas treated with the test article, Johnson's Baby Bar (comparator), had decreases of 9 to 15 opacity units from the PE opacity to the final opacity determination.

For regulatory purposes, the *In Vitro* Irritation Score (IVIS) cut-off values for identifying test chemicals as inducing serious eye damage (UN GHS Category I) and test chemicals not requiring classification for eye irritation or serious eye damage (UN GHS No Category) are found in the table below (OECD 437, adopted 26 July 2013).

For non-regulatory purposes, the following classification system was established by Sina et al.[2] based on studies with a wide range of test materials. While this classification system provides a good initial guide to interpretation of these *in vitro* data, these specific ranges are not applicable to all classes of materials or other exposure times. Whenever possible, results should be compared to "benchmark" materials tested under similar exposure conditions.

In Vitro Score:

≤ 25 = mild irritant
from 25.1 to 55 = moderate irritant
from 55.1 and above = severe irritant

Table 1
BCOP Results of the Test Articles and the Positive Control

Sponsor's Designation	Conc. (w/v)	Exposure Time	Opacity Value	OD490 Value	*In Vitro* Score	pH
Formulation of the invention	10%	10 minutes	0.9	0.039	1.4	10.5
Johnson's Baby Bar (comparator), Formula# e0043-011, Batch# e0043-011	10%	10 minutes	2.3	0.139	4.4	10.5
Ethanol	NA	10 minutes	34.0	0.970	48.6	NA

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CLAIMS

1. A bar soap composition providing an effective antimicrobial benefit against pathogens such as gram positive / gram negative bacteria and viruses, which bar soap compositions comprises:

a) up to 10%wt. of a non-quaternary ammonium based germicidal compound,

b) at least 45wt% of salts of C_{12}, C_{14} ,C_{16} and C_{18} saturated fatty acids,

c) at least 20wt% water,

d) up to 10%wt. of secondary alkane sulfonate,

which is substantially free of silicate based fillers.

2. A composition according to claim 1, wherein the non-quaternary ammonium based germicidal compound is present at a level of 0.1 to 5wt%, preferably about 1wt%.

3. A composition according to claim 2, wherein the non-quaternary ammonium based germicidal compound comprises chloroxylenol and / or triclocarban.

4. A composition according to claim 1, 2 or 3, wherein the bar soap composition is characterized in exhibiting a reduction of *Klebsiella pneumonia*, *S. aureus* and *E. coli*

5. A composition according to claim 4, wherein the bar soap composition is characterized in exhibiting at least a 1.5 log 10 reduction of *Klebsiella pneumonia*, *S. aureus* and *E. coli* when tested according to the standardized test protocols of ASTM E 1 053 Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces.

6. A composition according to any one of claims 1-5, wherein the composition comprises a fragrance.

7. A method for providing a germicidal benefit to a topical surface, especially a dermal surface, the method comprising the step of: contacting a topical surface upon which the presence of one or more undesired pathogens, preferably bacteria, are known or suspected, with a bar soap composition according to any one of claims 1-6.

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2015/053444

A. CLASSIFICATION OF SUBJECT MATTER
INV. C11D3/48 C11D9/00 C11D10/04
ADD. C11D1/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C11D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/065538 A1 (UNILEVER PLC [GB]; UNILEVER NV [NL]; LEVER HINDUSTAN LTD [IN]) 14 June 2007 (2007-06-14) page 4, lines 14-25 page 5, lines 14-23 page 7, lines 16-19 page 11, line 26 - page 12, line 3 example 1 claims 1, 5-8	1-7
X	US 6 218 348 B1 (ARONSON MICHAEL PAUL [US] ET AL) 17 April 2001 (2001-04-17) column 2, line 52 - column 3, line 11 column 4, lines 11-47 column 5, line 65 - column 6, line 16 column 6, line 56 - column 7, line 20 examples 4, 7, 8; tables 10-12	1-7

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 January 2016	29/01/2016

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2015/053444

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2013/144603 A1 (RECKITT & COLMAN OVERSEAS [GB]) 3 October 2013 (2013-10-03) page 1, lines 13-20 page 2, lines 7-31 page 8, lines 28-30 page 12, lines 16-24 examples; tables 1, 2 -----	1-7

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2015/053444

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007065538 A1	14-06-2007	AU 2006322383 A1	14-06-2007
		BR PI0617130 A2	12-07-2011
		CA 2619348 A1	14-06-2007
		EP 1957623 A1	20-08-2008
		US 2009286706 A1	19-11-2009
		WO 2007065538 A1	14-06-2007
		ZA 200802444 A	30-09-2009
US 6218348 B1	17-04-2001	NONE	
WO 2013144603 A1	03-10-2013	NONE	