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(71) Applicant: **THE PROCTER & GAMBLE COMPANY**
 [US/US]; One Procter & Gamble Plaza, Cincinnati, Ohio
 45202 (US).

(72) Inventors; and

(71) Applicants (*for US only*): **WEI, Karl Shiqing** [US/US];
 One Procter & Gamble Plaza, Cincinnati, Ohio 45202 (US).
ZHAO, He [CN/SG]; 70 Biopolis Street, 138547 Singapore
 (SG). **XU, Jian** [CN/CN]; No. 189 of Songling Rd, Qing-
 dao, Shandong 266101 (CN).

(74) Agent: **SHANGHAI PATENT & TRADEMARK LAW
 OFFICE, LLC**; 435 Guiping Road, Shanghai 200233
 (CN).

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(54) Title: METHODS OF IMPROVING SKIN APPEARANCE

	Rinse-Off			Leave-On	
	BCP2	B7U	BCP2+ZPT	Vehicle	Niacinamide
Adj. Mean ChgBsln SCORAD Week 4	-15.3	-15.0	-15.4	-14.4	-18.1
Adj. Mean 4 Week Steroid Use (g)	6.7	10.1	5.6	4.4	3.4
	(*sig. vs. B7U)		(*sig. vs. B7U)		

- Moisturizing products drive significantly lower steroid usage.

Fig. 7

(57) Abstract: Materials and methods for improving the appearance of a subject with an amenable skin with a composition comprising a zinc compound, a biocompatible surfactant and a lipid are provided. Amenable skin includes healthy skin and skin exhibiting atopic dermatitis (with or without lesions), skin dysbiosis and/or acne.



METHODS OF IMPROVING SKIN APPEARANCE**FIELD**

[0001] The disclosure relates to the fields of cosmetic and therapeutic applications to mammalian skin.

BACKGROUND

[0002] Metal-containing compounds such as metal pyrithiones, including zinc pyrithione, are known to exhibit antimicrobial activity. Zinc pyrithione is used as a bioactive component of shampoos useful in the treatment of dandruff through an anti-fungal effect on skin fungi. Independently, a variety of surfactants are known to be useful in removing skin microbes and particulate matter (i.e., dirt) from skin and hair in washing formulations, and surfactants in other formulations are known to be used as excipients serving as a wetting agent or stabilizer of particulate compositions, but providing no bioactivity (WO 2014/195872). Published PCT patent application WO 03/007900 discloses the use of lipophilic agents, such as ceramides, along with zinc pyrithione to increase the level of lipids in the skin and in scalp for treatment of dandruff. Some formulations combine agent classes, but remain focused on developing formulations for the simple cleaning of skin or for treating dry skin or dandruff.

[0003] Formulations useful in washing skin, treating dandruff, or treating dry skin, however, do not provide compounds or compositions useful in improving the quality and appearance of skin that is healthy or is associated with a skin condition other than dry skin or dandruff. Accordingly, a need continues to exist for materials, *e.g.*, formulations of bioactives, and methods for improving the quality and appearance of skin that is healthy or is associated with a skin condition such as atopic dermatitis (with or without lesions), skin dysbiosis or acne.

SUMMARY

[0004] The disclosure provides materials and methods for selectively improving the appearance of an amenable skin, *i.e.*, healthy skin or skin with atopic dermatitis (with or without lesions), skin dysbiosis, and/or acne. The disclosure provides a composition comprising a zinc compound (*e.g.*, a zinc ionophore such as zinc pyrithione), a biocompatible surfactant and a lipid. Application of compositions according to the disclosure to the skin results in the surprisingly selective inhibition of the *Staphylococcus* genus including, *e.g.*, *S. aureus* and *S. epidermidis*, while enhancing the viability, and thus presence, of other bacterial genera (*e.g.*, *Streptococcus*, *Propionibacterium*, *Corynebacterium*, *Micrococcus*). As a result of the composition-induced alteration in skin microbiota composition, the quality and

appearance of healthy skin is significantly improved, and the quality, appearance and health of skin with atopic dermatitis (with or without lesions), skin dysbiosis, or acne is significantly improved.

[0005] One aspect of the disclosure provides a method of improving the appearance of amenable skin comprising administering to a portion of the skin a rinse-off multi-phase skin improvement composition comprising a cleanser comprising a zinc compound and a lathering biocompatible surfactant, and a benefit agent comprising a lipid. Suitable zinc compounds include zinc monoglycerolate or a zinc ionophore, such as zinc pyrithione or zinc pyridinethione. For example, 0.1-2.0 μg zinc pyrithione may be administered per cm^2 skin. Surfactants suitable for use in the methods of the disclosure include a non-ionic surfactant or an anionic surfactant. By way of example, the anionic surfactant can be sodium trideceth(n) sulfate, where n is from 0.5 to 2.7. Suitable lipids for use in the methods include petrolatum, glyceryl monooleate, glycerin, ceramide, cholesterol, a fatty acid, a triglyceride, a phospholipid, or any combination thereof, such as a combination of petrolatum and glyceryl monooleate in a petrolatum:glyceryl monooleate ratio of about 98:2 by weight. For example, the lipid can be 5% glycerin. In any of these methods, the disclosure contemplates that 20-200 μg lipid is administered per cm^2 skin. The method may further comprise administering 5% niacinamide per cm^2 skin. In some exemplary methods, the composition is administered to the skin for at least three, four, or twenty-four hours, such as wherein the composition is administered daily for at least two, three, or four weeks.

[0006] In methods of the disclosure, by comparison to the skin prior to administration of the composition of the disclosure, the improved skin appearance results from any one or more of the following skin changes: reduced skin irritation, reduced average skin lesion size, reduced skin lesion number, reduced skin dryness, increased resistance to skin transepithelial water loss, reduced total protein level in the stratum corneum layer of skin, reduced involucrin level in the skin, reduced HSA level in the skin, reduced lipid level in the skin, reduced amino acid level in the skin, reduced hyaluronic acid level in the skin, reduced urea level in the skin, reduced linoleic acid level in the skin, reduced glycosaminoglycan level in the skin, reduced glycerin level in the skin, reduced mucopolysaccharide level in the skin, reduced pyrrolidone carboxylic acid (PCA) level in the skin, increased differentiation of keratins 1, 10 and 11 in the skin, reduced IL-1 α level in the skin, reduced IL-1R α level in the skin, reduced IL-8 level in the skin, or a reduced level of histamine in the skin. In exemplary methods, the improved skin appearance results from modification of the bacterial composition of a skin microbiota relative to the bacterial composition of the skin microbiota

prior to administration of the composition. For example, the skin microbiota of the skin of improved appearance may comprise a reduced level of *Staphylococcus* relative to the skin level of *Staphylococcus* prior to administration of the multi-phase skin improvement composition. The *Staphylococcus* may be any *Staphylococcal* species, such as *Staphylococcus aureus* or *Staphylococcus epidermidis*. The microbiota of the skin of improved appearance may comprise an increased level of at least one of *Propionibacterium*, *Corynebacterium*, or *Streptococcus* relative to the skin prior to administration of the multi-phase skin improvement composition, and this relative increase in level may be accompanied by a decrease in the level of *Staphylococcus* on the skin.

[0007] Another aspect of the disclosure is drawn to a method of assessing the improvement in skin appearance of a subject comprising: (a) obtaining a first measure of a skin biomarker; and (b) obtaining a second measure of a skin biomarker, wherein the first measures of a skin biomarker is obtained before the second measure of a biomarker is obtained, and wherein the skin biomarker is transepithelial water loss; total protein in the stratum corneum; involucrin; human serum albumin (HSA); skin lipid; skin amino acids; skin PCA; skin lactic acid; degree of differentiation among Keratins 1, 10 and 11; IL-1 α , IL-1ra, or IL-8; or skin histamine. In practicing the methods, skin appearance is improving if the second measure of the biomarker relative to the first measure of the biomarker is lower when the biomarker is transepithelial water loss, total stratum corneum protein, level of Involucrin in the skin, HSA level in the skin, IL-1 α level in the skin, IL-1ra level in the skin, IL-8 level in the skin, or histamine level in the skin. Skin appearance is improving if the second measure of the biomarker relative to the first measure of the biomarker is higher when the biomarker is skin lipid, skin amino acids, skin pyrrolidone carboxylic acid, skin lactic acid, or the degree of differentiation of Keratin 1, Keratin 10 and Keratin 11 in the skin.

[0008] Another aspect of the disclosure is a method of assessing the risk of developing a skin disorder comprising measuring the relative abundance of at least one bacterial genus on the skin, wherein the risk of developing a skin disorder increases if the relative abundance of at least one of *Staphylococcus*, *Paenibacillus*, *Microbacterium*, *Dermacoccus*, or *Propionibacterium* increases, or if at least one of *Streptococcus*, *Corynebacterium*, *Paracoccus*, *Kocuria*, *Deinococcus*, *Actinomyces*, *Neisseria*, or *Rothia* decreases, or any combination thereof. In such a method, the relative abundance of at least one bacterial genus may be determined by comparing the abundance of that bacterial genus to its abundance on the skin of a healthy control. Skin subjected to the method may comprise a skin lesion, or may exhibit a skin disorder such as atopic dermatitis, skin dysbiosis or acne.

[0009] Yet another aspect of the disclosure provides a method of assessing the risk of developing a skin disorder comprising measuring the change in bacterial diversity of the skin microbiota relative to the bacterial diversity of a healthy skin microbiota, wherein the risk of developing a skin disorder increases with decreasing bacterial diversity relative to the diversity of a healthy skin microbiota. In methods according to this aspect of the disclosure, the change in bacterial diversity is measured by a change in the Shannon Index, such as wherein the change in bacterial diversity is measured by at least a 20% change in the Shannon Index. In exemplary methods, at least a 20% change in the Shannon Index is indicative of an increased risk of developing a skin disorder if the Shannon Index decreases by at least 20%, reflective of a decrease in biodiversity, as opposed to the Shannon Index increasing by at least 20%, indicative of an increase in skin microbiota diversity, which is associated with improved skin appearance. In exemplary methods, the risk of developing a skin disorder in the form of atopic dermatitis, skin dysbiosis or acne is assessed.

[0010] Aspects of the disclosure are also described in the following enumerated paragraphs.

1. A method of improving the appearance of amenable skin comprising administering to a portion of the skin a rinse-off multi-phase skin improvement composition comprising a cleanser comprising a zinc compound and a lathering biocompatible surfactant, and a benefit agent comprising a lipid.

2. The method of paragraph 1 wherein the zinc compound is zinc monoglycerolate or a zinc ionophore.

3. The method of paragraph 2 wherein the zinc ionophore is zinc pyrithione or zinc pyridinethione.

4. The method of any of the preceding enumerated paragraphs wherein 0.1-2.0 μg zinc pyrithione is administered per cm^2 skin.

5. The method of any of the preceding enumerated paragraphs wherein the surfactant is a non-ionic surfactant or an anionic surfactant.

6. The method of paragraph 5 wherein the anionic surfactant is sodium trideceth(n) sulfate, where n is from 0.5 to 2.7.

7. The method of any of the preceding enumerated paragraphs wherein the lipid is petrolatum, glyceryl monooleate, glycerin, ceramide, cholesterol, a fatty acid, a triglyceride, a phospholipid, or any combination thereof.

8. The method of any of the preceding enumerated paragraphs wherein the lipid comprises a combination of petrolatum and glyceryl monooleate in a petrolatum:glyceryl monooleate ratio of about 98:2 by weight.
9. The method of any of paragraphs 1-7 wherein the lipid is 5% glycerin.
10. The method of any of the preceding enumerated paragraphs wherein 20-200 μg lipid is administered per cm^2 skin.
11. The method of any of the preceding enumerated paragraphs further comprising administering 5% niacinamide per cm^2 skin.
12. The method of any of the preceding enumerated paragraphs wherein the composition is administered to the skin for at least three, four, or twenty-four hours.
13. The method of any of the preceding enumerated paragraphs wherein the composition is administered daily for at least two, three, or four weeks.
14. The method of any of the preceding enumerated paragraphs wherein, compared to the skin prior to administration of the composition, the improved skin appearance results from reduced skin irritation, reduced average skin lesion size, reduced skin lesion number, reduced skin dryness, increased resistance to skin transepithelial water loss, reduced total protein level in the stratum corneum layer of skin, reduced involucrin level in the skin, reduced HSA level in the skin, reduced lipid level in the skin, reduced amino acid level in the skin, reduced hyaluronic acid level in the skin, reduced urea level in the skin, reduced linoleic acid level in the skin, reduced glycosaminoglycan level in the skin, reduced glycerin level in the skin, reduced mucopolysaccharide level in the skin, reduced pyrrolidone carboxylic acid level in the skin, increased differentiation of keratins 1, 10 and 11 in the skin, reduced IL-1 α level in the skin, reduced IL-1R α level in the skin, reduced IL-8 level in the skin, or a reduced level of histamine in the skin.
15. The method of any of paragraphs 1-13 wherein the improved skin appearance results from modification of the bacterial composition of a skin microbiota relative to the bacterial composition of the skin microbiota prior to administration of the composition.

16. The method of paragraph 15 wherein the skin microbiota of the skin of improved appearance comprises a reduced level of *Staphylococcus* relative to the skin level of *Staphylococcus* prior to administration of the multi-phase skin improvement composition.

17. The method of paragraph 16 wherein the *Staphylococcus* is *Staphylococcus aureus* or *Staphylococcus epidermidis*.

18. The method of any of paragraphs 15-17 wherein the microbiota of the skin of improved appearance comprises an increased level of at least one of *Propionibacterium*, *Corynebacterium*, or *Streptococcus* relative to the skin prior to administration of the multi-phase skin improvement composition.

19. A method of assessing the risk of developing a skin disorder comprising measuring the relative abundance of at least one bacterial genus on the skin, wherein the risk of developing a skin disorder increases if the relative abundance of at least one of *Staphylococcus*, *Paenibacillus*, *Microbacterium*, *Dermacoccus*, or *Propionibacterium* increases, or if at least one of *Streptococcus*, *Corynebacterium*, *Paracoccus*, *Kocuria*, *Deinococcus*, *Actinomyces*, *Neisseria*, or *Rothia* decreases, or any combination thereof.

20. The method of paragraph 19 wherein the relative abundance of at least one bacterial genus is determined by comparing the abundance of that bacterial genus to its abundance on the skin of a healthy control.

21. The method of any of paragraphs 19-20 wherein the skin comprises a skin lesion.

22. A method of assessing the risk of developing a skin disorder comprising measuring the change in bacterial diversity of the skin microbiota relative to the bacterial diversity of a healthy skin microbiota, wherein the risk of developing a skin disorder increases with decreasing bacterial diversity relative to the diversity of a healthy skin microbiota.

23. The method of paragraph 22 wherein the change in bacterial diversity is measured by at least a 20% change in the Shannon Index.

[0011] 24. The method of any of paragraphs 19-23, wherein the skin disorder is atopic dermatitis, skin dysbiosis or acne.

[0012] Other features and advantages of the present disclosure will become apparent from the following detailed description, including the drawing. It should be understood, however, that the detailed description and the specific examples are provided for illustration only, because various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from the detailed description.

BRIEF DESCRIPTION OF THE DRAWING

[0013] Figure 1. Visual dryness of skin was assessed. Amenable human skin was subjected to the following daily treatments over a four-week period: (A) water, (B) BCP2 vehicle + zinc pyrithione, (C) BCP2 vehicle, (D) Cetaphil body wash, (E) B7U bar soap + Cetaphil LN, (F) SC99 vehicle (water (75-89% (wt. %)), glycerin (3-10%), Sepigel 305 thickener (1-2%), isopropyl isostearate (1%), isohexadecane (3%), cetyl alcohol (0.3%), stearyl alcohol (0.5%), dimethicone 9DC Q2-1503 (2%), ethylparaben (0.15%), propylparaben (0.07%), Pal-KT (0.0001-0.05%), and Glyco-repair™ (0.1-5.0%)) + 5% niacinamide (2-5%), or (G) SC99 vehicle. Results are shown as color-coded histograms measured three hours after a given daily application, with measurements taken on days 1 (*i.e.*, 1.3), 5 (*i.e.*, 5.3), 14 (*i.e.*, 14.3), 21 (*i.e.*, 21.3) and 28 (*i.e.*, 28.3). Lower case letters designate results that differ in a statistically significant manner, with intermediate results indicated by double lettering, e.g., results designated "a" differ significantly from results labeled "b", with intermediate results designated "ab". Statistical significance was set at $p = 0.05$ (2-sided) with a baseline of 2.998. All treatments delivered significant improvements relative to application of water, and all treatments virtually resolved the dryness issue by the end of the study. BCP2 vehicle products delivered benefits similar to Cetaphil lotion at 3 hours, and better improvement than Cetaphil body wash (BW) after four weeks of use (*i.e.*, three hours after the application on day 28) and during regression. All leave-on products provided superior performance at 24 hours.

[0014] Figure 2. Measurement of barrier function by trans-epithelial water loss (TEWL). A standard leg wash was used to measure the barrier function of amenable skin subjected to one of the following treatments over a period of daily treatments extending beyond three weeks: (A) water, (B) zinc pyrithione atopic dermatitis clinical, (C) atopic dermatitis clinical control, (D) Cetaphil body wash, (E) B7U bar soap with Cetaphil body moisturizer, (F) modified SC99HN + 5% niacinamide, or (G) modified SC99HN. Measurements of TEWL on day 2 provided baseline results. Readings after three weeks of daily treatments are shown in the color-coded histograms, with different lower case letters designating statistically significant differences in the results ($p=0.05$ (2-sided), with a baseline of 8.769. The results

show all leave-on products delivered superior performance compared to water and rinse-off treatments. Zinc pyrithione atopic dermatitis clinical, *i.e.*, zinc pyrithione in BCP2 vehicle showed lower, and thus better, TEWL after three weeks of daily treatments, compared to atopic dermatitis clinical control, Cetaphil body wash, and water. Zinc pyrithione in BCP2 vehicle consistently ranked between the leave-on products and all other rinse-off products at three and 24 hours post-treatment from week 2 onwards. Zinc pyrithione in BCP2 vehicle is better than water and Cetaphil body wash at 24 hours since week three, and BCP2 vehicle was better than Cetaphil body wash at three hours since week 3.

[0015] Figure 3. Figure 3 tabulates skin biomarkers useful in measuring and monitoring the appearance of amenable skin. Biomarkers useful in this context include total protein in the stratum corneum, involucrin, HSA, lipids, natural moisturizing factors (NMFs), Keratins 1, 10 and 11, cytokines and histamine. The Figure identifies endpoints for measurements, the rationale behind placing reliance on the biomarker, the expected outcome indicating benefit to the subject and the benefit perceived by the subject, or consumer.

[0016] Figure 4. Amenable skin was subjected to various treatments over a 32-day period to assess improvement in appearance by measuring a natural moisturizing factor (NMF) in the form of pyrrolidone carboxylic acid (*i.e.*, PCA). A standard leg wash was used to measure NMF (PCA) levels of amenable skin subjected to one of the following daily treatments over the study period: (A) water, (B) zinc pyrithione atopic dermatitis clinical, (C) atopic dermatitis clinical control, (D) Cetaphil body wash, (E) B7U bar soap with Cetaphil body moisturizer, (F) modified SC99HN + 5% niacinamide, or (G) modified SC99HN. Measurements of PCA at the initiation of the study provided baseline results. Readings after 14 days, 21 days, 28 days and 32 days of daily treatments are shown in the color-coded histograms, with different lower case letters designating statistically significant differences in the results ($p=0.05$ (2-sided), with a baseline of 516.753. The results showed that zinc pyrithione in BCP2 vehicle had significantly higher levels of PCA than the Cetaphil body wash control. In addition, zinc in BCP2 vehicle exhibited a directionally higher PCA when compared to BCP2 vehicle treatment. Higher levels of NMFs, including PCA, are associated with greater skin barrier integrity, a characteristic of skin of improved appearance.

[0017] Figure 5. The appearance of amenable skin subjected to various treatments was monitored by measuring Involucrin levels over a period of 32 days. A standard leg wash was used to measure Involucrin levels of amenable skin subjected to one of the following treatments over a period of daily treatments extending 32 days: (A) water, (B) zinc pyrithione atopic dermatitis clinical, (C) atopic dermatitis clinical control, (D) Cetaphil body wash, (E)

B7U bar soap with Cetaphil body moisturizer, (F) modified SC99HN + 5% niacinamide, or (G) modified SC99HN. Measurements of Involucrin levels at the initiation of the study provided baseline results. Readings after 14 days, 21 days, 28 days and 32 days of daily treatments are shown in the color-coded histograms, with different lower case letters designating statistically significant differences in the results ($p=0.05$ (2-sided), with a baseline of 0.002. The results reveal that zinc pyrithione in BCP2 vehicle yielded a lower level of Involucrin relative to the Cetaphil body wash control. Zinc pyrithione in BCP2 vehicle also had a directionally lower level of Involucrin relative to BCP2 vehicle alone (*i.e.*, AD Clinical Control). Lower levels of Involucrin are associated with skin of improved appearance relative to skin exhibiting relatively higher levels of Involucrin.

[0018] Figure 6. The appearance of amenable skin was assessed by monitoring the levels of inflammatory cytokines. Individuals were subjected to various treatments in a standard leg wash protocol extending over a 32-day period to assess improvement in appearance by determining the $\log_{10}$ of the ratio of IL-1ra (Interleukin 1 receptor antagonist) to IL-1 α (Interleukin 1 alpha). Subjects performed a daily leg wash involving one of the following treatments over the study period: (A) water, (B) zinc pyrithione atopic dermatitis clinical, (C) atopic dermatitis clinical control, (D) Cetaphil body wash, (E) B7U bar soap with Cetaphil body moisturizer, (F) modified SC99HN + 5% niacinamide, or (G) modified SC99HN. The $\log_{10}$ ratio of IL-1Ra:IL-1 α at the initiation of the study provided baseline results. Ratio determinations after 14 days, 21 days, 28 days and 32 days of daily treatments are shown in the color-coded histograms, with different lower case letters designating statistically significant differences in the results ($p=0.05$ (2-sided), with a baseline of 0.904. A lower $\log_{10}$ ratio of IL-1Ra:IL-1 α is associated with skin of improved appearance and the results showed that zinc pyrithione in BCP2 vehicle yielded a lower $\log_{10}$ ratio of IL-1Ra:IL-1 α than the Cetaphil body wash. Also, zinc pyrithione in BCP2 vehicle yielded a similar $\log_{10}$ ratio of IL-1Ra:IL-1 α compared to BCP2 vehicle alone.

[0019] Figure 7. Clinical study of amenable skin with atopic dermatitis (with lesions). Study design is described in Example 8. The results show the relative change in SCORAD (industry-accepted SCORing Atopic Dermatitis) from baseline to the conclusion of the study, revealing that rinse-off products, *i.e.*, zinc pyrithione in BCP2 vehicle, BCP2 vehicle, and B7U bar soap, led to improvements in atopic dermatitis over the seven-week study period, as measured by SCORAD. The improvement in atopic dermatitis was greater than achieved with the leave-on vehicle treatment, but less than the improvement in atopic dermatitis achieved with leave-on niacinamide. The Figure also shows that zinc pyrithione in BCP2

vehicle, and BCP2 vehicle, as rinse-off products yielded a significantly lower steroid usage to treat atopic dermatitis than use of B7U bar soap as a rinse-off product. The reduction in steroid usage achieved with zinc pyrithione in BCP2 vehicle as well as the reduction achieved with BCP2 vehicle alone were almost as significant as the reduction in steroid usage achieved with the leave-on vehicle or the leave-on niacinamide, providing promising results for the use of a zinc pyrithione-containing rinse-off product in improving the appearance of amenable skin.

DETAILED DESCRIPTION

[0020] The disclosure provides materials and methods benefiting man and other animals in improving the appearance of healthy skin and skin exhibiting atopic dermatitis (with or without lesions), skin dysbiosis or acne, *i.e.*, amenable skin conditions. Healthy skin is non-diseased skin, and it is also skin that does not appear to be dry and/or flaky, such as would be found in an individual with dandruff or with clinically dry skin. As such, methods of the disclosure are focused on methods of treating skin and not on methods of treating hair (*e.g.*, methods of delivering a bioactive to a hair follicle or infundibulum), or dandruff, and do not involve applications to clinically dry skin. Further, to maximize the availability and, hence, activity of the bioactive components of compositions according to the disclosure, *i.e.*, a zinc compound, a biocompatible surfactant and a lipid, the compositions and methods of use thereof involve the use of free bioactive compounds, *i.e.*, compounds not bound in polymeric form. Preferably, the compositions are also not caged in particulate forms. In general terms, the materials and methods of the disclosure improve the appearance of healthy skin and treat an amenable skin condition of a subject with atopic dermatitis (with or without lesions), skin dysbiosis and/or acne.

[0021] A detailed description of the various aspects of the disclosure will be more readily appreciated with an understanding of the following defined terms.

[0022] A "zinc-containing compound" or a "zinc compound" is any biologically compatible form of zinc resulting in the uptake of biologically active zinc such as simple zinc salts, *e.g.*, zinc oxide and zinc compounds including zinc monoglycerolate and zinc ionophores, such as zinc pyrithione and zinc pyridinethione. The various forms of zinc have been found to have beneficial effects on human skin beyond an anti-microbial action. In particular, zinc compositions have been found to improve the quality of healthy skin, *e.g.*, appearance. Accordingly, the zinc according to the disclosure is not in the form of a dried zinc-polymer aggregate. In other words, the zinc is not sequestered in particles comprising

other compounds such as polymers designed to enhance the anti-microbial properties of certain forms of zinc, such as zinc pyrithione.

[0023] A "biocompatible surfactant" of the disclosure is any surfactant known in the art to be useful in cleansing skin, including anionic, nonionic, zwitterionic or amphoteric surfactants, provided that the surfactant is also biocompatible, such as biocompatible surfactants like nonionic surfactants but also including sulfate-containing surfactants known to be biocompatible. In its role of cleansing skin, a biocompatible surfactant is defined as a bioactive compound, and not as an excipient. Consistent with this definition, a biocompatible surfactant refers to any mild surfactant or sulfate-containing surfactant that, in the amounts used in the compositions according to the disclosure, do not harm or otherwise have a deleterious effect on the skin. Biocompatible surfactants include anionic surfactants, including sulfate-containing surfactants, nonionic surfactants, amphoteric surfactants, zwitterionic surfactants, and mixtures thereof. Exemplary surfactants include sodium laureth(n) sulfate (SLEnS) surfactants.

[0024] The "lipid" according to the disclosure may be any lipid or lipid form known in the art. The lipid may be any of a variety of generally hydrophobic chemical compounds, but a lipid according to the disclosure is not a sphingolipid derivative or a wax. Exemplary lipids include petrolatum, glyceryl monooleate, glycerin, ceramide, cholesterol, a fatty acid, a triglyceride, a phospholipid, or any combination thereof.

[0025] "Amenable skin condition" means skin having atopic dermatitis without lesions, skin having atopic dermatitis with lesions, skin exhibiting skin dysbiosis, skin exhibiting acne, or healthy skin. An amenable skin condition is expressly defined to exclude skin dryness or skin characterized as exhibiting, or prone to, dandruff, or to skin infundibula, such as hair follicles.

[0026] "Biomarker" refers to any biological molecules (genes, proteins, lipids, metabolites) that can, singularly or collectively, reflect the current or predict future state of a biological system. Thus, as used herein, various biomarkers can be indicators of a quality of skin in terms of skin hydration, among several other properties. Non-limiting examples of biomarkers include inflammatory cytokines, natural moisturizing factors, one or more of keratins 1, 10 and 11, lipids and total protein. The response of skin to treatment with compositions, including personal care compositions for example, can be assessed by measuring one or more biomarkers.

[0027] "Multiphase" refers to compositions comprising at least two phases which can be chemically distinct (*e.g.*, a cleansing phase and a benefit phase). Such phases can be in direct physical contact with one another. A personal care composition can be a multiphase personal care composition where phases of the personal care composition can be blended or mixed to a significant degree, but still be physically distinct. In these situations, the physical distinctiveness is undetectable to the naked eye. The personal care composition can be a multiphase personal care composition where the phases are in physical contact and are visually distinct. Visually distinct phases can take many forms, for example, they can appear as striped, marbled, the like.

[0028] "Natural moisturizing factor (NMF)" refers to a collection of water-soluble compounds that can be found in the stratum corneum. These compounds comprise about 20-30% of the dry weight of the corneocyte. NMF components absorb water from the atmosphere and combine the water with NMF water content, allowing the outermost layers of the stratum corneum to stay hydrated despite being exposed to external elements. Such NMFs include amino acids, lactic acid, urea, and pyrrolidone carboxylic acid (PCA).

[0029] "Non-diseased skin" refers to skin that is generally free of disease, infection, and/or fungus. As used herein, dry skin is considered to be included in non-diseased skin.

[0030] "Dry skin" is usually characterized as rough, scaly, and/or flaky skin surface, especially in low humidity conditions and is often associated with the somatosensory sensations of tightness, itch, and/or pain.

[0031] "Package" refers to any suitable container for a personal care composition including but not limited to a bottle, tube, jar, non-aerosol pump, and combinations thereof.

[0032] "Personal care composition" refers to compositions intended for topical application to the skin. Personal care compositions can be rinse-off formulations, in which the product can be applied topically to the skin or hair and then subsequently rinsed within seconds to minutes from the skin with water. The product could also be wiped off using a substrate. In either case, it is believed at least a portion of the product is left behind (*i.e.*, deposited) on the skin. The personal care compositions can be extrudable or dispensable from a package. The personal care compositions can be in the form of, for example, a liquid, semi-liquid cream, lotion, gel, or a combination thereof. Examples of personal care compositions can include but are not limited to body wash, moisturizing body wash, shower gels, skin cleansers, cleansing milks, in shower body moisturizer, and cleansing compositions used in conjunction with a disposable cleansing cloth.

[0033] "Shannon Index" is given the meaning it has acquired in the art of a measure of

$$(H) = - \sum_{i=1}^s p_i \ln p_i$$

biodiversity. The Index is calculated using the formula Shannon Index

wherein p = proportion (n/N) of all individuals (N) in a sample that are a given species (n) and s is the number of species.

[0034] "STnS" refers to sodium trideceth(n) sulfate, wherein n can define the average number of moles of ethoxylate per molecule.

[0035] "Structured" refers to having a rheology that can confer stability on the personal care composition. A degree of structure can be determined by characteristics determined by one or more of the following methods: Young's Modulus Method, Yield Stress Method, or Zero Shear Viscosity Method or by a Ultracentrifugation Method, described in U.S. Pat. No. 8,158,566, granted on Apr. 17, 2012. A cleansing phase can be considered to be structured if the cleansing phase has one or more following characteristics: (a) Zero Shear Viscosity of at least 100 Pascal-seconds (Pa-s), at least about 200 Pa-s, at least about 500 Pa-s, at least about 1,000 Pa-s, at least about 1,500 Pa-s, or at least about 2,000 Pa-s; (b) A Structured Domain Volume Ratio as measured by the Ultracentrifugation Method, of greater than about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, or at least about 90%; or (c) A Young's Modulus of greater than about 2 Pascals (Pa), greater than about 10 Pa, greater than about 20 Pa, greater than about 30 Pa, greater than about 40 Pa, greater than about 50 Pa, greater than about 75 Pa, or greater than about 100 Pa.

[0036] "Lather" refers to an aerated foam which results from providing energy to aqueous surfactant mixtures, particularly dilute mixtures.

[0037] The phrase "substantially free of as used herein, unless otherwise specified means that the composition or method comprises less than about 5%, less than about 3%, less than about 1%, or even less than about 0.1% of the stated ingredient. The term "free of as used herein means that the composition or method comprises 0% of the stated ingredient that is the ingredient has not been added to the personal care composition. However, these ingredients may incidentally form as a byproduct or a reaction product of the other components of the personal care composition.

[0038] Disclosed herein is the surprising discovery that zinc-containing compounds, in combination with a biocompatible surfactant and a lipid in single-phase or multi-phase

applications to amenable skin, can provide the benefit of improving the appearance of healthy skin or improving a condition such as atopic dermatitis, skin dysbiosis or acne.

[0039] The compositions according to the disclosure include a zinc-containing compound, such as a zinc ionophore (*e.g.*, zinc pyrithione), a biocompatible surfactant and a lipid. These components of the compositions of the disclosure are described in greater detail below.

Zinc-Containing Compounds

[0040] A method of enhancing skin appearance can comprise applying a zinc-containing compound such as a zinc ionophore (*e.g.*, a zinc pyrithione or zinc pyridinethione) to the skin of an individual. Similarly, a method of treating an amenable skin condition, such as atopic dermatitis (with or without lesions), skin dysbiosis, or acne, can comprise applying a zinc-containing compound to the skin of an individual. Examples of such zinc-containing compounds include, for example, zinc salts. Examples of zinc salts useful herein include the following: zinc aluminate, zinc carbonate, zinc oxide, zinc phosphates, zinc selenide, zinc sulfide, zinc silicates, zinc silicofluoride, zinc borate, zinc hydroxide, zinc hydroxy sulfate, and combinations thereof.

[0041] The zinc-containing compound can comprise a zinc salt of 1-hydroxy-2-pyridinethione (known as "zinc pyrithione" or "ZPT"), for example, a mercaptopyridine-Noxide zinc salt. The ZPT can be made by reacting 1-hydroxy-2-pyridinethione (*i.e.*, pyrithione acid) or a soluble salt thereof with a zinc salt (*e.g.*, zinc sulfate) to form a zinc pyrithione precipitate, as illustrated in U.S. Pat. No. 2,809,971, and the zinc pyrithione can be formed or processed into platelet ZPT using, for example, sonic energy as illustrated in U.S. Pat. No. 6,682,724.

[0042] Zinc pyrithione can take the form of particulates, platelets, or a combination thereof. For example, where the zinc pyrithione is introduced as a particulate, such particulates may have an average particle size from about 0.1 pm to about 20 pm; such particulates may also have an average particle size from about 0.2 pm to about 10 pm.

[0043] Other non-limiting zinc-containing compounds include zinc-containing layered materials ("ZLM's"). Examples of zinc-containing layered materials useful herein include zinc-containing layered structures with crystal growth primarily occurring in two dimensions. It is conventional to describe layer structures as not only those in which all the atoms are incorporated in well-defined layers, but also those in which there are ions or molecules between the layers, called gallery ions (A. F. Wells "Structural Inorganic Chemistry" Clarendon Press, 1975). Zinc-containing layered materials (ZLM' s) may have zinc

incorporated in the layers and/or be components of the gallery ions. Many ZLMs occur naturally as minerals. Common examples include hydrozincite (zinc carbonate hydroxide), basic zinc carbonate, aurichalcite (zinc copper carbonate hydroxide), rosasite (copper zinc carbonate hydroxide) and many related minerals that contain zinc. Natural ZLMs can also occur wherein anionic layer species such as clay-type minerals (*e.g.*, phyllosilicates) contain ion-exchanged zinc gallery ions. All of these natural compounds can also be obtained synthetically or formed *in situ* in a composition or during a production process. Another common class of ZLMs that are often, but not always, synthetic, is layered double hydroxides, which are generally represented by the formula $[M^{2+}_{1-x}M^{3+}_x(OH)_2]^{x+} A^{m-}_{x/m+n} nH_2O$ and some or all of the divalent ions (M^{2+}) would be represented as zinc ions (Crepaldi, E L, Pava, P C, Tronto, J, Valim, J B J. Colloid Interfac. Sci. 2002, 248, 429-42).

[0044] Yet another class of ZLMs can be prepared and is called hydroxy double salts (Morioka, H., Tagaya, H., Karasu, M, Kadokawa, J, Chiba, K Inorg. Chem. 1999, 38, 4211-6). Hydroxy double salts can be represented by the general formula $[M^{2+}_{1-x}M^{2+}_{1+x}(OH)_{3(1-y)}]^{+} A^{n-}_{(1=3y)/n} nH_2O$ where the two metal ion may be different; if they are the same and represented by zinc, the formula simplifies to $[Zn_{1+x}(OH)_2]^{2x+} 2x A^{-} nH_2O$. This latter formula represents (where $x=0.4$) and contains common materials such as zinc hydroxychloride and zinc hydroxynitrate. These are related to hydrozincite wherein a divalent anion replaces the monovalent anion. These compounds can also be formed *in situ* in a composition or in or during a production process. These classes of ZLMs represent relatively common examples of the general category and are not intended to be limiting as to the broader scope of compounds that fit this definition.

[0045] Commercially available sources of basic zinc carbonate include Zinc Carbonate Basic (Cater Chemicals: Bensenville, Ill., USA), Zinc Carbonate (Shepherd Chemicals: Norwood, Ohio, USA), Zinc Carbonate (CPS Union Corp.: New York, N.Y., USA), Zinc Carbonate (Elementis Pigments: Durham, UK), and Zinc Carbonate AC (Bruggemann Chemical: Newtown Square, Pa., USA).

[0046] Basic zinc carbonate, which also may be referred to commercially as "Zinc Carbonate" or "Zinc Carbonate Basic" or "Zinc Hydroxy Carbonate", is a synthetic version consisting of materials similar to naturally occurring hydrozincite. The idealized stoichiometry is represented by $Zn_5(OH)_6(CO_3)_2$ but the actual stoichiometric ratios can vary slightly and other impurities may be incorporated in the crystal lattice.

[0047] Suitable examples of pyrithione compounds include zinc pyrithione, sodium pyrithione, pyrithione acid, dipyrithione, chitosan pyrithione, magnesium disulfide pyrithione, and combinations thereof. Pyrithione materials may also include other pyridinethione salts formed from heavy metals such as zinc, tin, cadmium, magnesium, aluminum, and zirconium.

[0048] To improve skin appearance and/or improve skin conditions amenable to treatment with the disclosed composition, a zinc-containing compound is applied to, and rinsed from, the skin of an individual at least once per day for several days. Skin treated with a zinc-containing compound of the disclosure can show improvements in, for example, the appearance of the skin. For example, a zinc-containing compound can be applied at least once per day for about 14 days or more; or at least once per day for about 21 days or more.

[0049] The zinc-containing compound can be applied directly to the skin or provided as part of a rinse-off personal care composition, which is further described herein. To achieve the enhanced appearance of the skin or improve skin comprising atopic dermatitis (with or without lesions), skin dysbiosis or acne, from about 0.1 ng/cm² to about 5.0 ng/cm²; from about 0.2 ng/cm² to about 5.0 ng/cm²; from about 0.5 ng/cm² to about 5.0 ng/cm²; from about 1.0 ng/cm² to about 3.0 ng/cm²; of a zinc-containing compound is deposited on the skin. Determination of the amount of zinc-containing compound deposited on the skin can be accomplished using techniques known in the art, such as the Cup Scrub method.

[0050] Improvements in skin appearance can be measured using known techniques, including, for example, a Corneometer. For example, typical Corneometer units range from about 15-20, wherein the higher the value the higher the level of skin moisturization; and the lower the value, the lower the level of moisturization. Methods for using a Corneometer are described below. Once a zinc-containing compound (*e.g.*, zinc pyrithione) is applied to an amenable skin surface of an individual, a measurement can be taken at predetermined time intervals to evaluate the effectiveness of the zinc-containing compound for improving the appearance of the skin, or for treating a skin with atopic dermatitis (with or without lesions), skin dysbiosis or acne.

[0051] For example, measurements taken 3 hours, 24 hours, or 48 hours after the zinc-containing compound has been applied to the skin demonstrate that zinc-containing compounds deposited on the skin can provide vast improvements to amenable skin conditions or to skin appearance. In fact, a Corneometer shows that about 3 hours after the 21st application of the zinc-containing compound to the skin there is readily detectable

improvement in skin appearance and/or an amenable skin condition (e.g., at least 0.3 Corneometer units).

[0052] Improvements in an amenable skin condition (atopic dermatitis (with or without lesions), skin dysbiosis, acne, or the appearance of healthy skin) can be measured and/or monitored through the use of biomarkers. In particular, natural moisturizing factors (NMFs) constitute an exemplary biomarker that can be detected through methods described herein. One suitable method of obtaining biological samples for measurement of skin NMFs is the application of tape to an epithelium. Any type of tape, including any type of medical tape, is suitable for use in obtaining biological samples of epithelia. This technique is well known in the art and is relatively simple to implement. The technique involves application of tape to the epithelial tissue, typically skin, followed by removal of the tape therefrom. The biomarker analytes obtained from the epithelial tissue and present on the tape can then be removed from the tape in any fashion that preserves the biomarker analytes for suitable detection and measurement assays. Suitable biomarkers and testing procedures for NMFs are described in U.S. patent application Ser. No. 13/007,630.

[0053] While improvements in skin condition can be measured using a Corneometer or biomarkers are exemplary approaches to measuring and/or monitoring an amenable skin condition such as atopic dermatitis, skin dysbiosis, acne, or the appearance of healthy skin, but other suitable measuring and monitoring methods are available that focus on other properties of amenable skin conditions. For example, change in an amenable skin condition can be measured and/or monitored by assessing the visual dryness of skin, trans-epidermal water loss (TEWL), total protein in the stratum corneum layer of skin, involucrin level in skin, HSA level in skin, lipid level in skin, relative differentiation of Keratins 1, 10 and 11 in skin, inflammatory cytokine (e.g., IL-1 α , IL-1 β , IL-8) level in skin, and histamine level in skin.

Biocompatible Surfactants

[0054] A method of enhancing skin appearance also comprises at least one biocompatible surfactant. The biocompatible surfactant can be anionic, non-ionic, amphoteric or zwitterionic, such as sodium laureth(n) sulfate, hereinafter SLEnS, wherein n defines the average moles of ethoxylation. In this context, the n variable can range from about 1 to about 3. Additional exemplary surfactants for use in the compositions of the disclosure include sodium lauroamphoacetate, sodium trideceth(n) sulfate, sodium chloride, fragrance (pashmina PCC), trideceth-3, methylchloroisothiazolinone, methylisothiazolinone, citric acid,

guar hydroxypropyltrimonium chloride, xanthan gum, sodium benzoate, PEG-90m, disodium EDTA, and sodium hydroxide.

[0055] The personal care composition can further comprise from about 0.1% to 20%, by weight of the personal care composition, of a co-surfactant. Co-surfactants according to the disclosure can comprise amphoteric surfactants, zwitterionic surfactants, or mixtures thereof. The rinse-off personal care composition can include at least one of an amphoteric surfactant and a zwitterionic surfactant. Suitable amphoteric or zwitterionic surfactants include those described in U.S. Pat. No. 5,104,646 and U.S. Pat. No. 5,106,609.

[0056] Amphoteric surfactants can include those that can be broadly described as derivatives of aliphatic secondary and tertiary amines in which an aliphatic radical can be straight or branched chain and wherein an aliphatic substituent can contain from about 8 to about 18 carbon atoms such that one carbon atom can contain an anionic water solubilizing group, *e.g.*, carboxy, sulfonate, sulfate, phosphate, or phosphonate. Examples of compounds falling within this definition can be sodium 3-dodecyl-aminopropionate, sodium 3-dodecylaminopropane sulfonate, sodium lauryl sarcosinate, N-alkyltaurines such as the one prepared by reacting dodecylamine with sodium isethionate according to U.S. Pat. No. 2,658,072, N-higher alkyl aspartic acids such as those produced according to U.S. Pat. No. 2,438,091, and products described in U.S. Pat. No. 2,528,378. Other examples of amphoteric surfactants can include sodium lauroamphoacetate, sodium cocoamphoacetate, disodium lauroamphoacetate disodium cocodiamphoacetate, and mixtures thereof. Amphoacetates and diamphoacetates can also be used.

[0057] Zwitterionic surfactants suitable for use can include those that are broadly described as derivatives of aliphatic quaternary ammonium, phosphonium, and sulfonium compounds, in which aliphatic radicals can be straight or branched chains, and wherein an aliphatic substituent can contain from about 8 to about 18 carbon atoms such that one carbon atom can contain an anionic group, *e.g.*, carboxy, sulfonate, sulfate, phosphate, or phosphonate. Other zwitterionic surfactants can include betaines, including cocoamidopropyl betaine.

Lipids

[0058] The compositions of the disclosure also comprise a lipid. Lipids according to the disclosure include any of a variety of natural or synthetic oils, fats or other generally hydrophobic compounds recognized in the art as lipids. Exemplary lipids include glycerides suitable for use as hydrophobic skin benefit agents, including castor oil, safflower oil, corn

oil, walnut oil, peanut oil, olive oil, cod liver oil, almond oil, avocado oil, palm oil, sesame oil, soybean oil, vegetable oils, sunflower seed oil, vegetable oil derivatives, coconut oil and derivatized coconut oil, cottonseed oil, derivatized cottonseed oil, jojoba oil, cocoa butter, petrolatum, mineral oil, and combinations thereof.

[0059] Non-limiting examples of alkyl esters suitable for use as lipid skin benefit agents herein include isopropyl esters of fatty acids and long chain esters of long chain (*i.e.*, C10-C24) fatty acids, *e.g.*, cetyl ricinoleate, nonlimiting examples of which include isopropyl palmitate, isopropyl myristate, cetyl riconoleate, and stearyl riconoleate. Other example include hexyl laurate, isohexyl laurate, myristyl myristate, isohexyl palmitate, decyl oleate, isodecyl oleate, hexadecyl stearate, decyl stearate, isopropyl isostearate, diisopropyl adipate, diisohexyl adipate, dihexyldecyl adipate, diisopropyl sebacate, acyl isononanoate lauryl lactate, myristyl lactate, cetyl lactate, and combinations thereof.

[0060] Non-limiting examples of alkenyl esters suitable for use as hydrophobic skin benefit agents herein include oleyl myristate, oleyl stearate, oleyl oleate, and combinations thereof.

[0061] Non-limiting examples of polyglycerin fatty acid esters suitable for use as lipid skin benefit agents include decaglyceryl distearate, decaglyceryl diisostearate, decaglyceryl monomyristate, decaglyceryl monolaurate, hexaglyceryl monooleate, and combinations thereof.

[0062] Non-limiting examples of lanolin and lanolin derivatives suitable for use as lipid skin benefit agents include lanolin, lanolin oil, lanolin wax, lanolin alcohols, lanolin fatty acids, isopropyl lanolate, acetylated lanolin, acetylated lanolin alcohols, lanolin alcohol linoleate, lanolin alcohol riconoleate, and combinations thereof.

[0063] Non-limiting examples of silicone oils suitable for use as lipid skin benefit agents include dimethicone copolyol, dimethylpolysiloxane, diethylpolysiloxane, mixed C1-C30 alkyl polysiloxanes, phenyl dimethicone, dimethiconol, and combinations thereof.

Nonlimiting examples of silicone oils useful herein are described in U.S. Pat. No. 5,011,681.

[0064] Still other suitable hydrophobic skin benefit agents include milk triglycerides (*e.g.*, hydroxylated milk glyceride) and polyol fatty acid olyesters.

Rinse-Off Personal Care Compositions

[0065] Zinc-containing compounds (*e.g.*, zinc pyrithione) can be applied to the skin through a rinse-off personal care composition. Suitable zinc-containing compounds are

disclosed hereinabove. A rinse-off personal care composition can be a single-phase composition or a multi-phase composition. The rinse-off personal care composition can also involve a single-phase application or a multi-phase application that includes a cleansing phase and a benefit phase. The cleansing phase and/or benefit phase can include the zinc-containing compound (*e.g.*, zinc pyrithione). The cleansing phase can also include, for example, various biocompatible surfactants as described herein. The benefit phase can comprise an effective amount of a lipid. The cleansing phase and the benefit phase can be blended, and/or patterned.

[0066] The rinse-off personal care composition can comprise at least about 0.1%, by weight of the rinse-off personal care composition, of a zinc-containing compound (*e.g.*, zinc pyrithione). The rinse-off personal care composition can also comprise from about 0.2% to about 1.0%, by weight of the rinse-off personal care composition, of a zinc-containing compound (*e.g.*, zinc pyrithione). The rinse-off personal care composition can also comprise about 0.5%, by weight of the rinse-off personal care composition, of a zinc-containing compound (*e.g.*, zinc pyrithione).

A. Cleansing Phase

[0067] A cleansing phase of a multi-phase composition according to the disclosure comprises a zinc-containing compound (*e.g.*, a zinc ionophore such as zinc pyrithione), as described herein. Further, the cleansing phase includes at least one biocompatible surfactant. For example, the cleansing phase can include an aqueous structured surfactant that is biocompatible. The concentration of the structured surfactant in the personal care composition can range from about 1% to about 20%, by weight; from about 2% to about 15%, by weight; and from about 5% to about 10%, by weight of the personal care composition.

[0068] Such a structured surfactant can include sodium trideceth(n) sulfate, hereinafter STnS, wherein n defines the average moles of ethoxylation. The n variable can range from about 0 to about 3. The n variable can also range from about 0.5 to about 2.7, from about 1.1 to about 2.5, from about 1.8 to about 2.2, or n can be about 2. When n can be less than 3, STnS can provide improved stability, improved compatibility of benefit agents within the rinse-off personal care compositions, and increased mildness of the rinse-off personal care compositions. These benefits of STnS are disclosed in U.S. Patent Application No. 13/157,665.

[0069] Further, the cleansing phase can comprise a structuring system, wherein the structuring system can comprise, optionally, a non-ionic emulsifier and an electrolyte. The rinse-off personal care composition can comprise at least a 70% lamellar structure.

[0070] Suitable surfactants or co-surfactants that can generally be used in a cleansing phase for a rinse-off personal care composition are described hereinabove and/or in McCutcheon's: Detergents and Emulsifiers North American Edition (Allured Publishing Corporation 1947) (1986), McCutcheon's, Functional Materials North American Edition (Allured Publishing Corporation 1973) (1992) and U.S. Pat. No. 3,929,678.

[0071] Other additives can optionally be included in the cleaning phase, including for example an emulsifier (*e.g.*, non-ionic emulsifier) and electrolytes. Suitable emulsifiers and electrolytes are described in U.S. patent application Ser. No. 13/157,665.

B. Benefit Phase

[0072] As noted herein, rinse-off personal care compositions can include a benefit phase. The benefit phase can be hydrophobic and/or anhydrous. The benefit phase can also be substantially free of surfactant.

[0073] The benefit phase can also include a benefit agent. In particular, the benefit phase can comprise from about 0.1% to about 50%, by weight of the rinse-off personal care composition, of the benefit agent. The benefit phase can also include from about 0.5% to about 20%, by weight of the rinse-off personal care composition, of the benefit agent. Examples of the benefit agent can include artificial sweat, castor oil, olive oil, oleic acid, Sefose® 1618S, Sefose® 1618U, petrolatum, glyceryl monooleate, mineral oil, natural oils (*e.g.*, soybean oil), and mixtures thereof. Other suitable benefit agents are described in U.S. patent application Ser. No. 13/157,665.

[0074] As noted herein, the benefit phase can include a zinc-containing and/or pyrithione material (*e.g.*, zinc pyrithione). Examples of such zinc-containing materials can include, for example, zinc salts. Examples of zinc salts useful can include the following: zinc aluminate, zinc carbonate, zinc oxide, zinc phosphates, zinc selenide, zinc sulfide, zinc silicates, zinc silicofluoride, zinc borate, zinc hydroxide, zinc hydroxy sulfate, and combinations thereof. The benefit phase can also include additional ingredients as described below.

[0075] The benefit phase can typically comprise one or more benefit agents, as set forth above. The benefit phase can comprise from about 0.1% to about 50%, by weight of the rinse-off personal care composition, of the benefit agent in the form of a lipid.

[0076] Additional ingredients can optionally be added to the rinse-off personal care composition for treatment of the skin, or to modify the aesthetics of the rinse-off personal care composition as is the case with perfumes, colorants, dyes or the like. Optional materials useful in products herein can be categorized or described by their cosmetic and/or therapeutic benefit or their postulated mode of action or function. However, it can be understood that actives and other materials useful herein can, in some instances, provide more than one cosmetic and/or therapeutic benefit or function or operate via more than one mode of action. Therefore, classifications herein can be made for convenience and cannot be intended to limit an ingredient to particularly stated application or applications listed. A precise nature of these optional materials, and levels of incorporation thereof, will depend on the physical form of the composition and the nature of the cleansing operation for which it is to be used. Optional materials can usually be formulated at about 6% or less, about 5% or less, about 4% or less, about 3% or less, about 2% or less, about 1% or less, about 0.5% or less, about 0.25% or less, about 0.1% or less, about 0.01% or less, or about 0.005% or less of the rinse-off personal care composition.

[0077] To further improve stability under stressful conditions such as high temperature and vibration, densities of separate phases can be adjusted such that they can be substantially equal. To achieve this, low density microspheres can be added to one or more phases of the rinse-off personal care composition. Examples of rinse-off personal care compositions that comprise low density microspheres are described in U.S. Patent Publication No. 2004/0092415A1.

[0078] Other non-limiting optional ingredients that can be used in the personal care composition can comprise an optional benefit component that can be selected from the group consisting of thickening agents; preservatives; antimicrobials; fragrances; chelators (*e.g.*, such as those described in U.S. Pat. No. 5,487,884 issued to Bisset, et al.); sequestrants; vitamins (*e.g.* Retinol); vitamin derivatives (*e.g.*, tocophenyl acetate, niacinamide, panthenol); sunscreens; desquamation actives (*e.g.*, such as those described in U.S. Pat. Nos. 5,681,852 and 5,652,228 issued to Bisset); anti-wrinkle/anti-atrophy actives (*e.g.*, N-acetyl derivatives, thiols, hydroxyl acids, phenol); anti-oxidants (*e.g.*, ascorbic acid derivatives, tocophenol) skin soothing agents/skin healing agents (*e.g.*, panthenoic acid derivatives, aloe vera, allantoin); skin lightening agents (*e.g.*, kojic acid, arbutin, ascorbic acid derivatives) skin tanning agents (*e.g.*, dihydroxyacetone); anti-acne medicaments; essential oils; sensates; pigments; colorants; pearlescent agents; interference pigments (*e.g.*, those disclosed in U.S. Pat. No. 6,395,691, U.S. Pat. No. 6,645,511, U.S. Pat. No. 6,759,376, U.S. Pat. No. 6,780,826) particles (*e.g.*, talc,

kolin, mica, smectite clay, cellulose powder, polysiloxane, silicas, carbonates, titanium dioxide, polyethylene beads) hydrophobically modified non-platelet particles (*e.g.*, hydrophobically modified titanium dioxide and other materials described in U.S. Pat. Pub. No. 2006/0182699A) and mixtures thereof. The multiphase personal care composition can comprise from about 0.1% to about 4%, by weight of the rinse-off personal care composition of hydrophobically modified titanium dioxide. Other such suitable examples of such skin actives are described in U.S. patent application Ser. No. 13/157,665.

[0079] Other optional ingredients can be most typically those materials approved for use in cosmetics and that are described in the CTFA Cosmetic Ingredient Handbook, Second Edition, The Cosmetic, Toiletries, and Fragrance Association, Inc. 1988, 1992.

Exemplary Procedures

A. Cup Scrub Procedure

[0080] As noted herein, the Cup Scrub Procedure can be used to assist in determining how much zinc-containing compound (*e.g.*, zinc ionophore such as zinc pyrithione) is deposited onto the skin of an individual. In performing the procedure, test subjects first wet the volar forearm surface under running water (flow=4.5 L/min, temp=35-38°C) for approximately 15 seconds. Next, test subjects receive a dose of 1 mL of body wash (via disposable syringe) to the volar forearm surface. The subjects proceed to generate lather on the volar forearm by rubbing the applied body wash with their opposite hand for approximately 15 seconds. Following the 15-second lathering process, the lather is allowed to sit undisturbed on the skin for an additional 15 seconds. At the end of the 15-second wait (30 seconds after the start of the lathering process), the subjects rinse the arm for approximately 10 seconds, allowing the running water to contact the proximal volar forearm surface and cascade down (toward the distal surface). Following the rinse, the subjects use a paper towel to pat the surface dry.

[0081] The next part of the procedure involves a 2-cm diameter glass cylinder containing a bead of silicone caulking on a skin contact edge that will be pressed firmly against a skin surface to prevent leakage of an extraction fluid. One mL of the extraction solvent can be pipetted into the glass cylinder. To determine how much zinc pyrithione is deposited, for example, the extraction solvent can be 80:20 0.05 M EDTA:Ethanol. While using a transfer pipette or glass rod, an entire area within the glass cylinder can be scrubbed for about 30 seconds using moderate pressure. The solution can be removed and pipetted into a labeled glass sample vial. The Cup Scrub Procedure can be repeated using fresh extraction solution, which will be pooled with the initial extraction in the labeled vial.

[0082] After each use, the glass cylinder and rod are cleaned. For example, each cylinder and rod can be immersed in dilute Dawn® solution and scrubbed with a finger or soft bristle brush. The cylinders and rods can then be immersed in isopropyl alcohol (IPA). Finally, cylinders and rods are wiped dry, *e.g.*, with a Kimwipe or other lint free tissue, to remove any visible residue. Scrub solutions can be changed at the end of each day or when any visible layer of residue can be found in the bottom thereof. Further, samples can be stored at 4°C ($\pm 3^\circ\text{C}$) until the samples are submitted for HPLC analysis. HPLC analysis is then used to determine the amount of deposition. The free pyrithione in solution is then derivatized with 2-2'-Dithiopyridine, and subsequently analyzed via HPLC utilizing UV detection. The results are reported as μg of zinc pyrithione per mL of solution.

B. Biomarkers: Natural Moisturizing Factors (NMFs)

[0083] Biomarkers that can be indicative of skin health can be measured to evaluate changes on one or more surfaces of epithelial tissue of a subject exposed to a product according to the disclosure. Thus, biomarkers can allow for a relatively simple, efficient and quick determination of the usefulness of a product for providing one or more benefits to skin, or for monitoring changes in the skin upon or after exposure of the skin to a composition according to the disclosure.

[0084] Samples of epithelial tissue can be obtained to collect and analyze biomarker analytes. Non-limiting examples of suitable techniques for obtaining samples include application of tape, rinsing by lavage, biopsy, swabbing, scraping, blotting and combinations thereof. Whichever technique is used to obtain a sample, it should be one where the biomarkers obtained are those present on the surface and/or in the epithelial tissue, and not those in any of the underlying non-epithelial tissue, such as muscle.

[0085] A method of obtaining epithelial tissue is by application of tape, such as, but not limited to, any type of medical tape. A technique for applying tape can be as straightforward as applying tape to the skin and then removing it. Biomarker analytes obtained from the skin and present on the tape can be removed from the tape by any technique known in the art that preserves the biomarker analytes for suitable detection and measurement assays. Examples of tapes can include, but are not limited to, D-squame Tape® and SEBUTAPE®, both of which are available from CuDerm Corporation, Dallas, Tex., USA; and Transpore® tape, which is available from the 3M company, of Minnesota USA.

[0086] Biomarker analytes can be present in test and control samples and can be identified using one or more techniques known in the art. Detection techniques such as antibody-based

binding methodologies, nucleotide probe-based specific hybridization assays, highly specific chemical tagging using markers, dyes, and other colorimetric and fluorometric probes and assays,, as well as enzyme-linked production of detectable labeled compounds can be used to detect and measure biomarker analytes. In some non-limiting examples, biomarker analytes include inflammatory cytokines, natural moisturizing factors (NMFs), keratin 1, keratin 10, keratin 11, lipids and total protein.

[0087] Examples of NMFs include amino acids, lactic acid, urea, and pyrrolidone carboxylic acid (PCA), and more particularly include Trans-Urocanic Acid, Citrulline, Glycine, Histidine, Ornithine, Proline, 2 Pyrrolidone 5 Acid, and Serine. As set forth above, effectiveness of treatment with a composition of the disclosure can be evidenced by an increase in the amount of NMFs. NMFs can be measured to detect improvement in skin appearance or reduction in the progression of an amenable skin condition in the form of atopic dermatitis (with or without lesions), skin dysbiosis or acne. Such methodologies are further described in U.S. Patent Application No. 13/007,630.

[0088] To measure NMF values, the following methods can be used. Tape strips (D-Squame) from subjects are placed into polypropylene tubes and mixed, by vortex or sonication, with acidified water to extract relevant amino acid-related NMFs (glycine, histidine, proline, serine, urocanic acid, citrulline ornithine and 2-pyrrolidone5-carboxylic acid). Extracts from the tape strips are spiked with stable-isotope internal standards of each NMF and then analyzed by gradient reversed-phase high-performance liquid chromatography with tandem mass spectrometry using multiple-reaction-monitoring. Combined standards for the NMFs are prepared over the required concentration range, spiked with the stable-isotope internal standards, and analyzed along with the samples. The response ratio of each standard (response of standard/response of internal standard) for each NMF is plotted versus the standard concentration to generate a regression curve for each of the NMFs. The concentration of each NMF in the extracts is then determined by interpolation from the appropriate regression standard curve.

[0089] The following examples illustrate exemplary methods of the disclosure. Example 1 provides an analysis of the skin microbiota of a subject with atopic dermatitis. Example 2 discloses a comparison of the skin microbiotas of a subject with atopic dermatitis and a healthy subject. Example 3 shows the changes in diversity of skin microbiotas as healthy skin develops atopic dermatitis. Example 4 discloses a model for predicting the development of an amenable skin condition such as atopic dermatitis and Example 5 reveals the

measuring/monitoring of changes in the skin microbiota as an amenable skin condition such as atopic dermatitis is treated with a composition according to the disclosure.

EXAMPLES

Example 1

Microbiota analysis of the skin of a subject with atopic dermatitis

[0090] In the following Examples, various skin biomarkers are assessed for their utility in assessing and monitoring the health and appearance of mammalian, *e.g.*, human, skin. The experiments disclosed in the Examples below show that a composition according to the disclosure comprising BCP2 vehicle + zinc ionophore (zinc pyrithionate) + lipid significantly improved both barrier function and barrier integrity, in particular by comparison to commercially available Cetaphil body wash. Cetaphil body wash comprises water, *Butyrospermum parkii* (Shea) butter, sodium trideceth sulfate, glycerin, *Helianthus annuus* (Sunflower) seed oil, sodium chloride, sodium lauroamphoacetate, cocamide MEA, niacinamide, tocopheryl acetate, allantoin, arginine, citric acid, methylisothiazolinone, sodium PCA, 1,2-hexanediol, caprylyl glycol, guar hydroxypropyltrimonium chloride, potassium sorbate, and disodium EDTA. BCP2 vehicle comprises cocoamidopropyl betaine (2.45%), sodium chloride (4.03%), trideceth-3 (1.31%), guar hydroxypropyltrimonium chloride (0.43%), xanthan gum (0.19%), acrylate/C10-C30 alkyl acrylates cross-polymer (0.03%), sodium benzoate (0.27%), citric acid (50% in DI water to pH=5.7), preservative (0.04%), perfume (0.25%), and water and minors (Q.S.); where appropriate, zinc pyrithione (0.5%), sodium trideceth-2 sulfate (8.21%), petrolatum (14.70%), and glyceryl monooleate (0.30%) can be included. Further, this composition of BCP2 vehicle + zinc ionophore (zinc pyrithionate) + lipid significantly enhanced Natural Moisturizing Factor (NMF) levels in the skin, comparing favorably to, *e.g.*, Cetaphil body wash in having a greater increase in NMF than the commercially available product. Additionally, the composition according to the disclosure performed better than Cetaphil body wash in significantly reducing skin irritation.

Example 2

Effect of compositions on skin with atopic dermatitis (without lesions)

[0091] A comparative study was conducted to assess the beneficial effect of compositions according to the disclosure on amenable skin such as the skin of a subject with lesion-free atopic dermatitis. The effects of compositions according to the disclosure were compared to water, a commercially available body wash (Cetaphil RestoraDerm) and leave-on lotions

(SC-99 with or without niacinamide and Cetaphil RestoraDerm). Subjects examined included those with healthy skin, those with sound, but unhealthy, skin in the form of atopic dermatitis without lesions, and sound, but unhealthy, skin in the form of atopic dermatitis with lesions. In the various comparative studies, measurements were taken with a corneometer, near infrared light, trans-epidermal water loss (TEWL), confocal RAMAN, pH, and biomarkers. Microbiota characterization included determination of bacterial composition and bacterial counts. Severity of atopic dermatitis was determined using SCORAD (SCORing of Atopic Dermatitis). Subjects were also scored for QOL (Quality Of Life).

[0092] More particularly, a standard Leg Controlled Application Test (LCAT) transient washing assay was conducted with subjects divided into seven groups and exposed to one of the following seven treatments: A - Negative control: water application (no treatment); B - Test product: vehicle (BCP2) + 0.5% zinc ionophore (zinc pyrithionate) +15% PET/GMO; C - Test product: vehicle (BCP2) + 0.5% zinc ionophore (zinc pyrithionate); D - Competitive moisturizer: B7U bar soap (no zinc ionophore) + Cetaphil lotion; E - Competitive body wash: Cetaphil body wash; F - Test product: SC-99 + 5% glycerin + 5% niacinamide; and G - Vehicle control: SC-99 + 5% glycerin. Subjects had atopic dermatitis without visible lesions. For each treatment, dryness of leg skin was visually assessed daily over a four-week period. Results shown in Figure 1 (treatments identified below each histogram bar) revealed that all tested treatments delivered significant improvement in comparison to water (negative control) and each treatment virtually resolved the skin dryness by the end of the four-week study. Further, BCP2 products delivered similar benefits to those delivered by Cetaphil lotion at 3 hours and greater improvement than Cetaphil body wash at 3 hours after four weeks of use (*i.e.*, three hours after the application applied at the four-week mark), as well as during regression. Leave-on products yielded superior performance to rinse-off products (*e.g.*, body wash) at 24 hours. Different lower-case letters above histogram bars identify treatments having statistically different effects on dryness ($p = 0.05$; 2-sided test). Thus, "a" was statistically different from "b", which was significantly different from "c", which statistically differed from "d", with the greatest difference between "a" and "d". The baseline value determined from the data was 2.998.

Example 3

Trans-epithelial water loss

[0093] Another comparative study was conducted to measure the flux of water (aqua flux) across the skin by assessing trans-epithelial water loss (TEWL) in a three-week study of subjects with atopic dermatitis without lesions. Treatments assessed using a standard LCAT

were: A - water only; B - AD (atopic dermatitis) Clinical + 0.5% zinc pyrithionate as a zinc ionophore; C - AD Clinical Control; D - Cetaphil Body Wash; E - B7 Body Moisturizer; F - Mod SC99HN + 5% Niacinamide; and G - Mod SC99HN No Niacinamide. Results provided in Figure 2 establish that leave-on products delivered superior performance (*i.e.*, greater TEWL reduction) compared to water or rinse-off treatments. Also, BCP2 with a zinc ionophore (zinc pyrithionate) performed better than body wash without a zinc ionophore, water, or Cetaphil body wash three hours after the application applied after three weeks of use. BCP2 with a zinc ionophore (zinc pyrithionate) also ranked consistently between all other rinse-off treatments and the leave-on products at three hours and 24 hours from week 2 onwards. Various treatments identified above by capital letter are indicated below histogram bars in Figure 2. Different lower-case letters above histogram bars identify treatments having statistically different effects on dryness ($p = 0.05$; 2-sided test). Thus, "a" was statistically different from "b", which was significantly different from "c", which statistically differed from "d", with the greatest difference between "a" and "d". The baseline value determined from the data was 8.769.

Example 4

Skin biomarkers

[0094] A variety of skin biomarkers were also investigated to determine their relevance to predicting or determining skin conditions such as atopic dermatitis, skin dysbiosis, or acne. The skin biomarkers are identified in Figure 3.

[0095] One desired property characteristic of healthy skin is the cohesiveness of the stratum corneum layer of the skin. One biomarker useful for assessing stratum corneum cohesiveness is the total protein in the stratum corneum. The reason for investigating total protein in the stratum corneum is that total protein is lower and exhibits more cohesiveness in healthy stratum corneum and higher total protein and less cohesiveness in sound, but unhealthy, skin with total protein steadily increasing and cohesiveness steadily decreasing with increasing skin damage. A beneficial treatment of less than healthy skin is expected to yield a reduction in total protein in the stratum corneum layer of the skin.

[0096] Barrier integrity is also a significant feature of healthy skin, and three biomarkers have been identified as providing information useful in assessing this feature. Involucrin and HSA levels in the skin are lower in healthy skin and higher in sound, but unhealthy, skin as well as in unsound skin. A beneficial skin treatment is expected to reduce the level of involucrin and HSA in the skin.

[0097] Lipid levels are also useful in interrogating barrier integrity. Exemplary lipids are ceramides, fatty acids, and cholesterol. Lipid levels are relatively higher in healthy skin in comparison to sound, but unhealthy, skin or unsound skin. Beneficial skin treatment is expected to increase the level of lipids in the skin.

[0098] Natural moisturizing factors (i.e., NMFs) are relatively high in healthy skin, with lower levels in sound, but unhealthy, skin or in unsound skin. Treatments providing beneficial effects to the skin are expected to increase the level of NMFs in the skin.

[0099] The levels of cell differentiation in the skin is also a feature of healthy skin that has been found to vary as skin becomes sound, but unhealthy, or becomes unsound. More particularly, the relative level of progenitor differentiation into the various keratin cell types, i.e., Keratin 1, 10 and 11, has been found to be correlated to the relative health of skin, with greater differentiation into Keratins 1, 10 and 11 occurring in healthy skin as opposed to sound, but unhealthy, skin or unsound skin.

[00100] The level of irritation and/or inflammation in skin is also related to skin health. Higher levels in skin irritation and/or inflammation are associated with increasingly unhealthy skin. Biomarkers for skin irritation and/or inflammation include the inflammatory cytokines and their cognate receptors, including IL-1 α , IL-1 β and IL-8. A beneficial skin treatment is expected to lower the levels of inflammatory cytokines and/or their cognate receptors, thereby reducing skin irritation and/or inflammation.

[00101] Yet another hallmark of healthy skin is the relative absence of itch. The itch sensation increases with increasingly unhealthy skin. A biomarker that correlates with skin itch is histamine level, which is elevated in increasingly unhealthy skin. A beneficial skin treatment is expected to lower the level of histamine in subjects experiencing itch, thereby providing relief.

Example 5

Effects of treatments on NMFs

[0100] A comparative study was conducted to examine the effects of various skin treatments on the level of natural moisturizing factors (NMFs) in subjects with atopic dermatitis without lesions. The study used a standard LCAT format for application of one of the following seven treatments: A - water; B - AD Clinical + zinc ionophore (zinc pyrithionate); C - AD Clinical control (no zinc ionophore); D - Cetaphil body wash; E - B7U bar soap + Cetaphil moisturizer; F - Mod SC99HN + 5% Niacinamide; and G - Mod SC99HN No Niacinamide. Results are provided in Figure 4, with each treatment assessed at

baseline, 14.0 days, 21.0 days, 28.0 days and 32.0 days, as shown by the grouped histogram bars in the Figure. Measures of pyroglutamic acid (PY acid), also known as pyrrolidone carboxylic acid (PCA), are normalized ($\log_{10}$) and are indicated by the heights of individual histogram bars at each time point, with the color of the bar identifying the treatment being assessed. Accordingly, higher measures are associated with healthier skin. Different lower-case letters above histogram bars identify treatments having statistically different effects ($p = 0.05$; 2-sided test). Thus, "a" was statistically different from "b", which was significantly different from "c", which statistically differed from "d", with the greatest difference between "a" and "d". The baseline value determined from the data was 516.753. The results demonstrably reveal that BCP2 + zinc ionophore (zinc pyrithionate) had a significantly higher PCA levels than the Cetaphil control. Additionally, BPC2 vehicle + zinc ionophore (zinc pyrithionate) had directionally higher PCA levels in comparison to treatment with the BCP2 vehicle alone.

Example 6

Effect of treatments on Involucrin

[0101] Effects of various skin treatments on skin involucrin levels was also investigated in subjects with atopic dermatitis without lesions. The study design involved the use of a routine LCAT and the following treatment protocols were examined: A - water; B - AD Clinical + zinc ionophore (zinc pyrithionate); C - AD Clinical control (no zinc ionophore in the form of zinc pyrithionate); D - Cetaphil body wash; E - B7U bar soap + Cetaphil moisturizer; F - Mod SC99HN + 5% Niacinamide; and G - Mod SC99HN No Niacinamide. Measures of Involucrin levels were normalized ($\log_{10}$) to total skin protein and are indicated by the heights of individual histogram bars at each time point, with the color of the bar identifying the treatment being assessed (see Figure 5). Accordingly, lower measures are associated with healthier skin. Different lower-case letters above histogram bars identify treatments having statistically different effects ($p = 0.05$; 2-sided test). Thus, "a" was statistically different from "b", which was significantly different from "c", which statistically differed from "d", with the greatest difference between "a" and "d". The baseline value determined from the data was 0.002. The data establish that BCP2 vehicle + zinc ionophore (zinc pyrithionate) yielded a lower level of Involucrin than Cetaphil body wash. BPC2 vehicle + zinc ionophore (zinc pyrithionate) exhibited a directionally lower level of Involucrin compared to BCP2 vehicle without a zinc ionophore (zinc pyrithionate).

Example 7*Effect of treatments on inflammatory cytokines*

[0102] A comparative experiment was performed to study the effects of various skin treatments on the level of inflammatory cytokines in the skin in subjects with atopic dermatitis without lesions. The study used a standard LCAT format for application of one of the following seven treatments: A - water; B - AD Clinical + zinc ionophore (zinc pyrithionate); C - AD Clinical control (no zinc ionophore); D - Cetaphil body wash; E - B7U bar soap + Cetaphil moisturizer; F - Mod SC99HN + 5% Niacinamide; and G - Mod SC99HN No Niacinamide. Results are provided in Figure 6, with the inflammatory cytokine levels measured as the ratio of IL-1 α :IL-1 α ($\log_{10}$). Each treatment was assessed at baseline, 14.0 days, 21.0 days, 28.0 days and 32.0 days, as shown by the grouped histogram bars in Figure 6. Different lower-case letters above histogram bars identify treatments having statistically different effects ($p = 0.05$; 2-sided test). Thus, "a" was statistically different from "b", which was significantly different from "c", which statistically differed from "d", with the greatest difference between "a" and "d". Results were significant at the .05 level; the baseline value determined from the data was 0.904. Apparent from the data in Figure 6 is that the treatment with BCP2 vehicle + zinc ionophore (zinc pyrithionate) yielded a lower ratio of IL-1 α :UK-1 α , and thus had a more beneficial effect on sound, but unhealthy, skin of subjects with atopic dermatitis without lesions, in comparison to treatment of such subjects with a Cetaphil body wash. Moreover, the results obtained with BCP vehicle + zinc ionophore (zinc pyrithionate) were approximately the same as treatment with BCP2 vehicle alone.

Example 8*Effect of treatments on atopic dermatitis (with lesions)*

[0103] A comparative clinical study was conducted to assess the effects of various rinse-off treatments on atopic dermatitis subjects with at least two lesions. The study design involved a seven-week randomized, double-blind, parallel group study of in-home use of various treatments comprising a one-week washout period, a four-week treatment period involving a single daily application of 0.1% hydrocortisone butyrate to lesions in addition to the experimental treatment, and a two-week regression period. Study subjects were males and females of 4-18 years of age having Fitzpatrick skin Type I-IV, with approximately 28 subjects for each of the tested treatments. Treatments groups involved daily application of a B7U bar soap as a basic control group, daily application of BCP2 vehicle + 15%

petrolatum/glyceryl monooleate (PET/GMO) as one test group and BCP2 vehicle + 15% PET/GMO + 0.5% zinc ionophore (zinc pyrithionate). Measurements were taken at baseline and at weeks 2, 4 and 6. The results of the phase I and phase II clinical studies are presented in Figure 7 in terms of SCORAD values, a scoring system for atopic dermatitis well-known in the art. The results show that the BCP2 vehicle alone has a more beneficial effect on atopic dermatitis skin with lesions than the B7U bar soap, with BPC2 vehicle + zinc ionophore (zinc pyrithionate) exhibiting a superior effect on atopic dermatitis with lesions compared to either BCP2 vehicle alone or B7U bar soap. All three rinse-off treatments had a beneficial effect, to one degree or another, on atopic dermatitis skin with lesions relative to a generic vehicle used as a leave-on treatment. A leave-on treatment with 5% niacinamide had a greater beneficial effect on atopic dermatitis skin with lesions than any of the rinse-off treatments, but that effect comes at the cost of living with an application left on the skin for considerable time periods, which leads to progressively deteriorating compliance with the treatment regimen. Also notable in the data presented in Figure 7 is that the rinse-off treatment with BCP2 vehicle alone or with BCP2 vehicle + zinc ionophore (zinc pyrithionate) had the beneficial effect of driving significantly lower steroid usage, with BCP2 vehicle + zinc ionophore (zinc pyrithionate) driving lower levels of steroid usage than rinse-off treatment with BCP2 vehicle alone.

[0104] Each of the references cited herein is incorporated by reference herein in its entirety, or in relevant passage, as would be apparent from the context of its citation. The disclosed subject matter has been described with reference to various specific examples and techniques. It should be understood, however, that many variations and modifications may be made while remaining within the spirit and scope of the disclosed subject matter.

CLAIMS

What is claimed is:

1. A method of improving the appearance of amenable skin comprising administering to a portion of the skin a rinse-off multi-phase skin improvement composition comprising a cleanser comprising a zinc compound and a lathering biocompatible surfactant, and a benefit agent comprising a lipid.
2. The method of claim 1 wherein the zinc compound is zinc monoglycerolate or a zinc ionophore.
3. The method of claim 2 wherein the zinc ionophore is zinc pyrithione or zinc pyridinethione.
4. The method of any of the preceding claims wherein 0.1-2.0 μg zinc pyrithione is administered per cm^2 skin.
5. The method of any of the preceding claims wherein the surfactant is a non-ionic surfactant or an anionic surfactant.
6. The method of claim 5 wherein the anionic surfactant is sodium trideceth(n) sulfate, where n is from 0.5 to 2.7.
7. The method of any of the preceding claims wherein the lipid is petrolatum, glyceryl monooleate, glycerin, ceramide, cholesterol, a fatty acid, a triglyceride, a phospholipid, or any combination thereof.
8. The method of any of the preceding claims wherein the lipid comprises a combination of petrolatum and glyceryl monooleate in a petrolatum:glyceryl monooleate ratio of about 98:2 by weight.
9. The method of any of claims 1-7 wherein the lipid is 5% glycerin.
10. The method of any of the preceding claims wherein 20-200 μg lipid is administered per cm^2 skin.
11. The method of any of the preceding claims further comprising administering 5% niacinamide per cm^2 skin.

12. The method of any of the preceding claims wherein the composition is administered to the skin for at least three, four, or twenty-four hours.

13. The method of any of the preceding claims wherein the composition is administered daily for at least two, three, or four weeks.

14. The method of any of the preceding claims wherein, compared to the skin prior to administration of the composition, the improved skin appearance results from reduced skin irritation, reduced average skin lesion size, reduced skin lesion number, reduced skin dryness, increased resistance to skin transepithelial water loss, reduced total protein level in the stratum corneum layer of skin, reduced involucrin level in the skin, reduced HSA level in the skin, reduced lipid level in the skin, reduced amino acid level in the skin, reduced hyaluronic acid level in the skin, reduced urea level in the skin, reduced linoleic acid level in the skin, reduced glycosaminoglycan level in the skin, reduced glycerin level in the skin, reduced mucopolysaccharide level in the skin, reduced pyrrolidone carboxylic acid level in the skin, increased differentiation of keratins 1, 10 and 11 in the skin, reduced IL-1 α level in the skin, reduced IL-1R α level in the skin, reduced IL-8 level in the skin, or a reduced level of histamine in the skin.

15. The method of any of claims 1-13 wherein the improved skin appearance results from modification of the bacterial composition of a skin microbiota relative to the bacterial composition of the skin microbiota prior to administration of the composition.

16. The method of claim 15 wherein the skin microbiota of the skin of improved appearance comprises a reduced level of *Staphylococcus* relative to the skin level of *Staphylococcus* prior to administration of the multi-phase skin improvement composition.

17. The method of claim 16 wherein the *Staphylococcus* is *Staphylococcus aureus* or *Staphylococcus epidermidis*.

18. The method of any of claims 15-17 wherein the microbiota of the skin of improved appearance comprises an increased level of at least one of *Propionibacterium*, *Corynebacterium*, or *Streptococcus* relative to the skin prior to administration of the multi-phase skin improvement composition.

19. A method of assessing the risk of developing a skin disorder comprising measuring the relative abundance of at least one bacterial genus on the skin,

wherein the risk of developing a skin disorder increases if the relative abundance of at least one of *Staphylococcus*, *Paenibacillus*, *Microbacterium*, *Dermacoccus*, or *Propionibacterium* increases, or if at least one of *Streptococcus*, *Corynebacterium*, *Paracoccus*, *Kocuria*, *Deinococcus*, *Actinomyces*, *Neisseria*, or *Rothia* decreases, or any combination thereof.

20. The method of claim 19 wherein the relative abundance of at least one bacterial genus is determined by comparing the abundance of that bacterial genus to its abundance on the skin of a healthy control.

21. The method of any of claims 19-20 wherein the skin comprises a skin lesion.

22. A method of assessing the risk of developing a skin disorder comprising measuring the change in bacterial diversity of the skin microbiota relative to the bacterial diversity of a healthy skin microbiota, wherein the risk of developing a skin disorder increases with decreasing bacterial diversity relative to the diversity of a healthy skin microbiota.

23. The method of claim 22 wherein the change in bacterial diversity is measured by at least a 20% change in the Shannon Index.

24. The method of any of claims 19-23, wherein the skin disorder is atopic dermatitis, skin dysbiosis or acne.

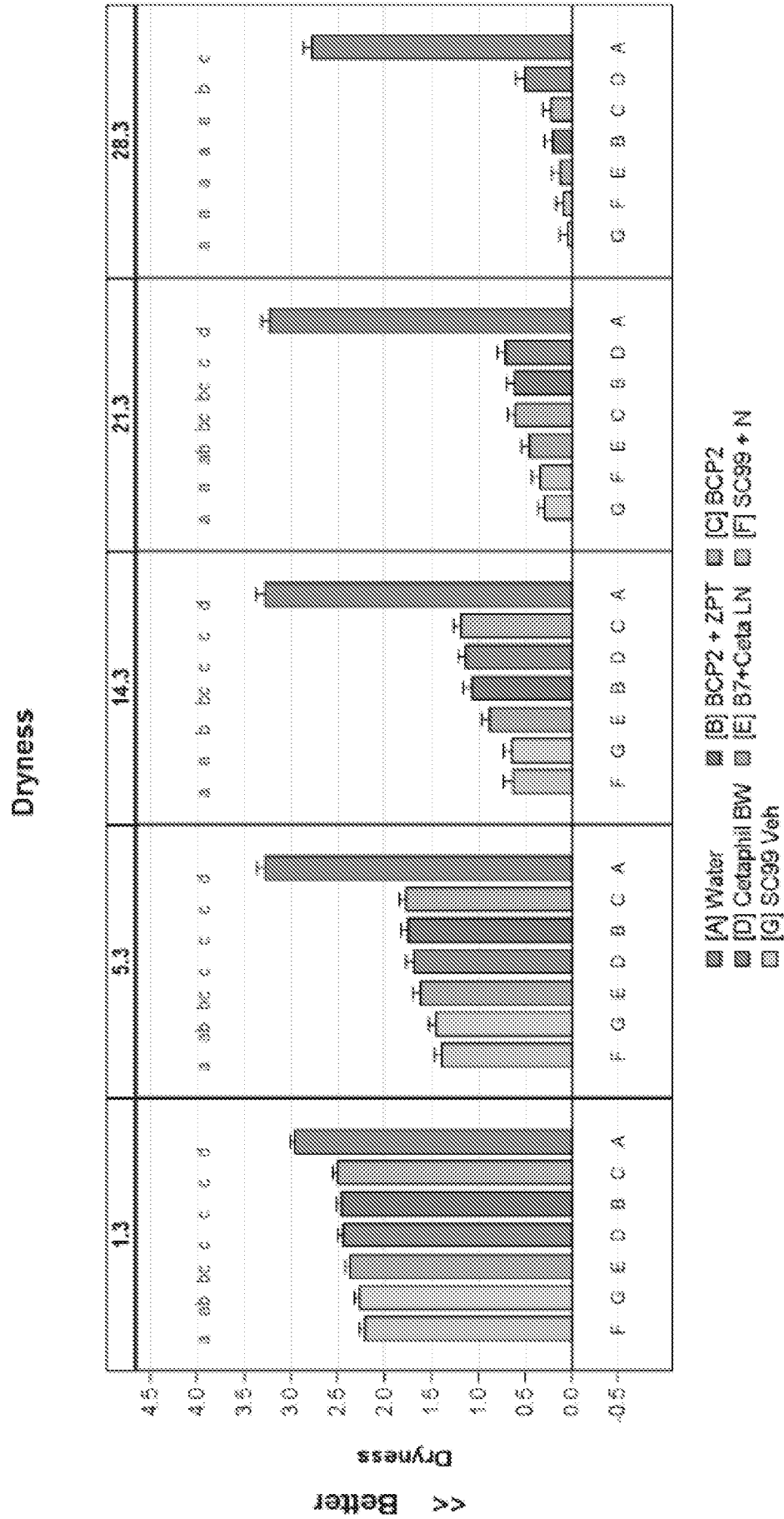


Fig. 1

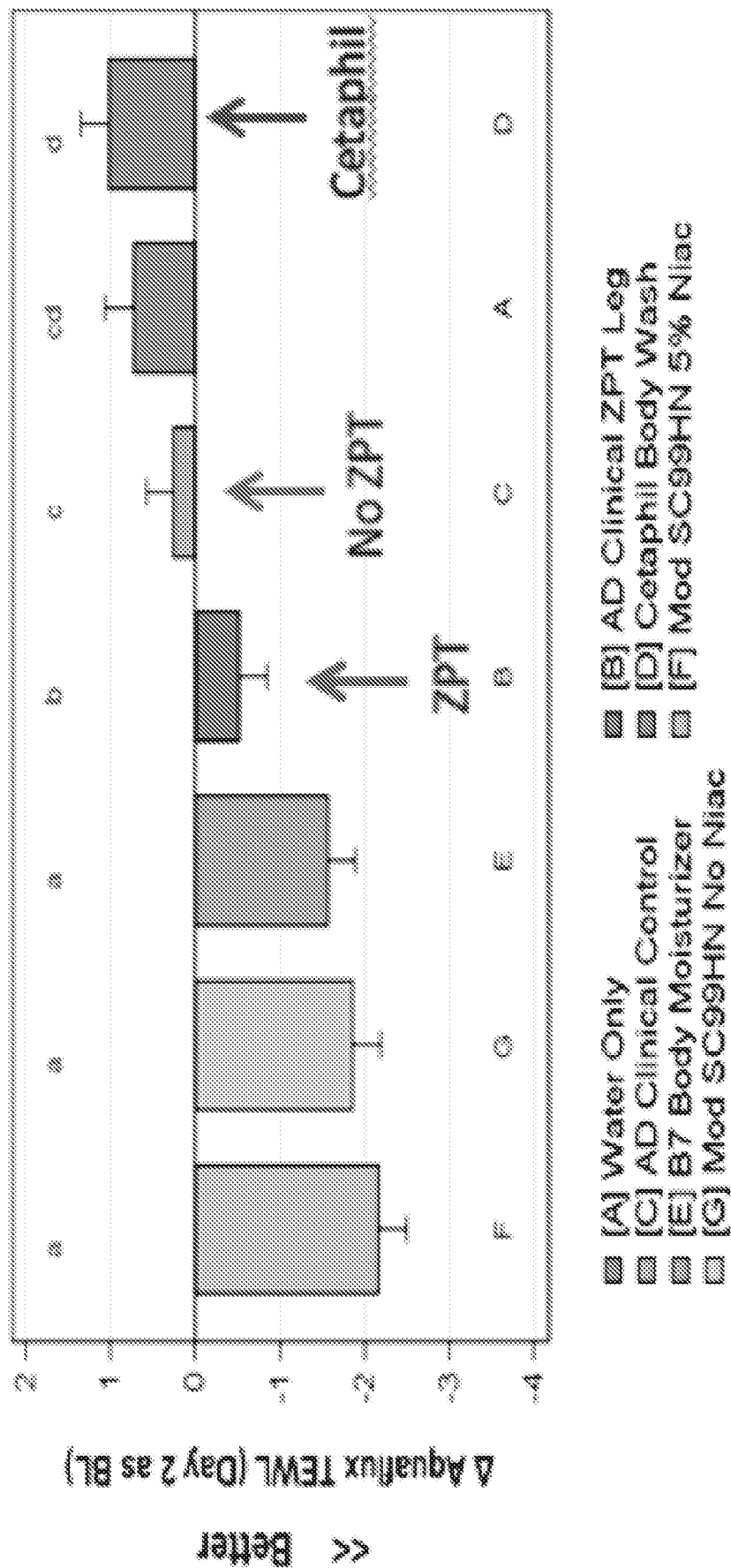


Fig. 2

Endpoints	Biomarkers	Rationale	Expected Outcome for Benefit	Consumer Benefits
Stratum Corneum Cohesiveness	Total Protein	• More cohesiveness in healthy stratum corneum. Less cohesiveness in damaged skin.	↓	• Reducing Dry Skin Flakes
Barrier Integrity	Involucrin and HSA	• Low level in healthy skin. Elevated in diseased skin.	↓	• Increasing Moisture • Improving skin health.
	Lipids (ceramides, fatty acids, cholesterol)	• High level in healthy skin. Low level in damaged skin.	↑	
	NMFs (amino acids, PCA, lactic acid)	• High level in healthy skin. Low level in damaged skin.	↑	
Differentiation	Keratin 1, 10 11	• Higher differentiation in normal/healthy skin.	↑	• Increasing Moisture • Improving skin health.
Irritation/ Inflammation	Cytokines IL-1 α , IL-1 β , IL-8	• Elevated in diseased/irritated skin.	↓	• Reducing skin irritation.
Itch	Histamine	• Elevated in diseased/irritated skin.	↓	• Providing itch relief.

Fig. 3

Higher is better

PY ACID Normalized (Log10)

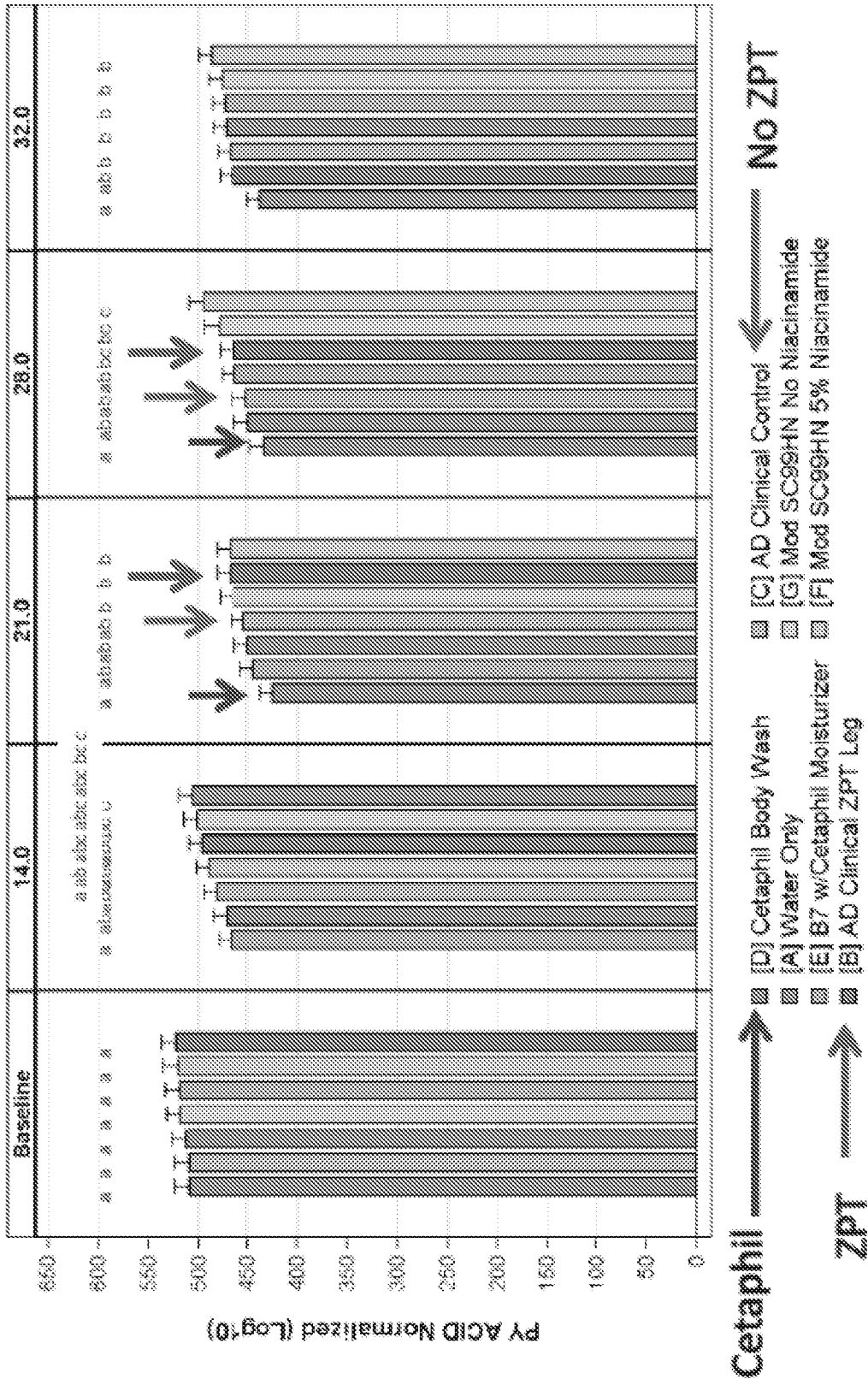


Fig. 4

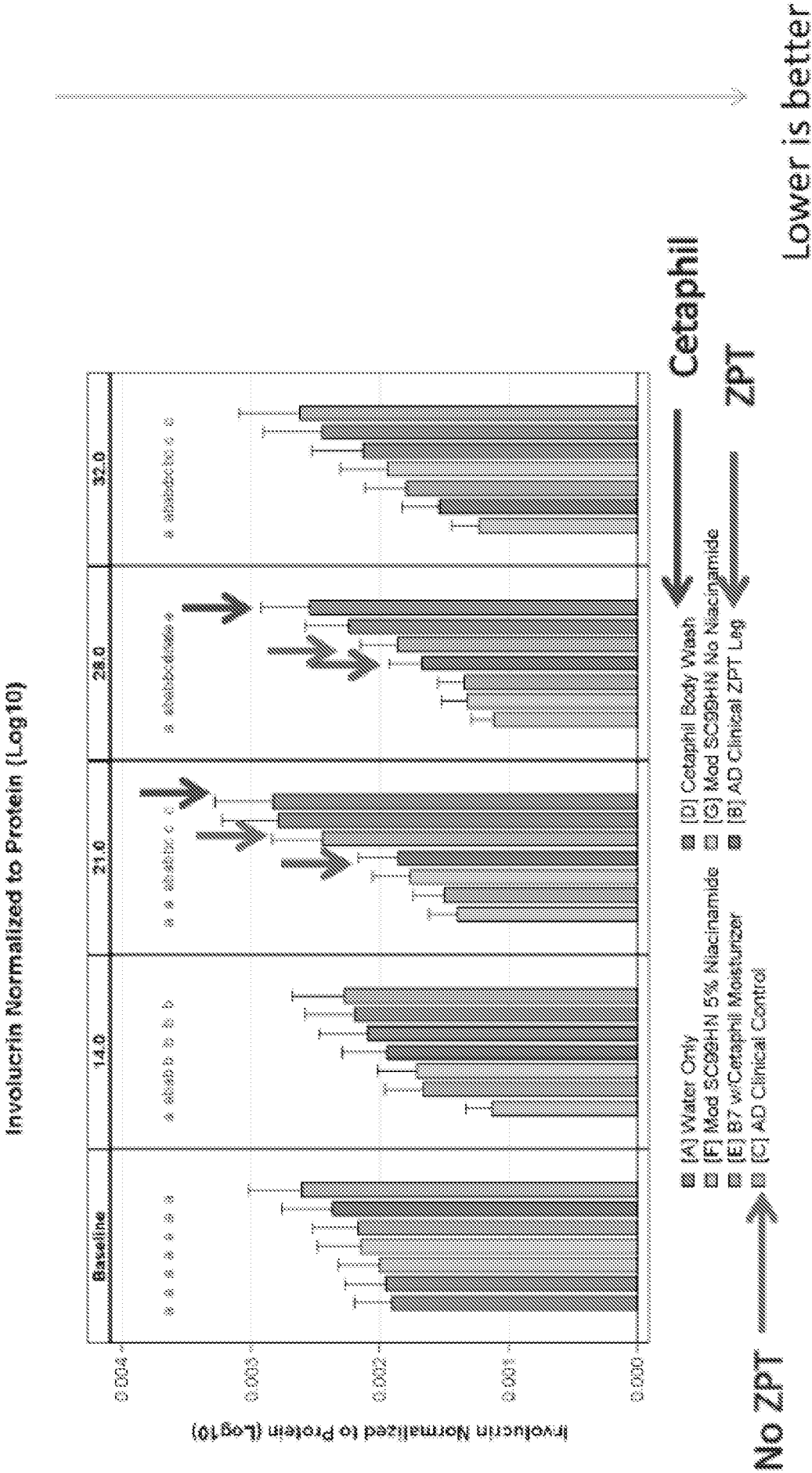


Fig. 5

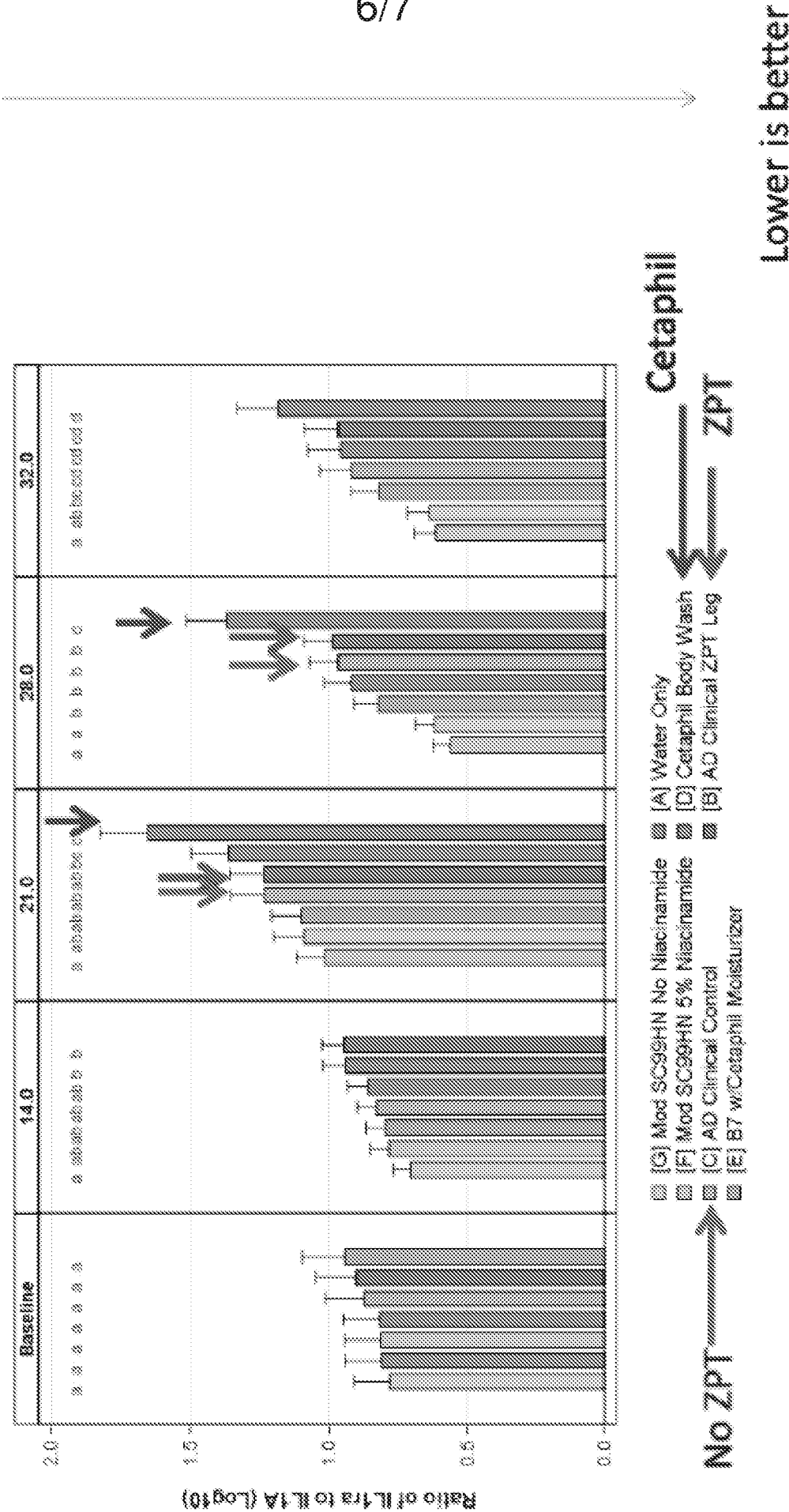


Fig. 6

	-----Rinse-Off-----			-----Leave-On-----		
	BCP2	B7U	BCP2+ZPT	Vehicle	Niacinamide	
Adj. Mean ChgBsln SCORAD Week 4	-15.3	-15.0	-15.4	-14.4	-18.1	
Adj. Mean 4 Week Steroid Use (g)	6.7 (*sig. vs. B7U)	10.1	5.6 (*sig. vs. B7U)	4.4	3.4	

- Moisturizing products drive significantly lower steroid usage.

Fig. 7

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2016/098822

A. CLASSIFICATION OF SUBJECT MATTER

A61K 8/27(2006.01)i; A61K 8/31(2006.01)i; A61P 17/00(2006.01)i; A61Q 19/00(2006.01)i; A61K 31/355(2006.01)i

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)

A61K A61P A61Q

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)

WPI, EPODOC, CNKI, CNPAT, NCBI PubMed, GOOGLE Scholar, ISI Web of Knowledge, STN, PROCTER & GAMBLE, zinc monoglycerolate, zinc ionophores, zinc pyrithione, zinc pyridinethione, anionic, nonionic, zwitterionic, amphoteric surfactants, sodium laureth sulfate, lipid, petrolatum, glyceryl monooleate, glycerin, ceramide, cholesterol, fatty acid, triglyceride, phospholipid, BCP2 vehicle, organic chelant, surfactant, EDTA, fatty acid, triglyceride, phospholipid, acne, atopic dermatitis, dysbiosis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 104981232 A (PROCTER & GAMBLE CO) 14 October 2015 (2015-10-14) , claim 1; paragraphs 23 and 83; example C	1-18
X	CN 105101948 A (PROCTER & GAMBLE CO) 25 November 2015 (2015-11-25) , claims 1-24; paragraphs 78 and 147-157	1-18
E	WO 2016179446 A1 (PROCTER & GAMBLE CO) 10 November 2016 (2016-11-10) , claims 1-15	1-18
X	WANG Shan et al. "The Update Progress of relationship between Skin Microbiome and Atopic Dermatitis," <i>China Medical Abstract of Dermatology</i> , Vol. 33, No. 2, 30 April 2016 (2016-04-30), , lines 1-4 of right column, lines 28-34 of left column, page 125	19-24

☐ Further documents are listed in the continuation of Box C.☒ 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

08 May 2017

Date of mailing of the international search report

02 June 2017

Name and mailing address of the ISA/CN

STATE INTELLECTUAL PROPERTY OFFICE OF THE
P.R.CHINA
6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing
100088
China

Authorized officer

TU, Haihua

Facsimile No. (86-10)62019451

Telephone No. (86-10)62413749

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2016/098822

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: **1-24**
because they relate to subject matter not required to be searched by this Authority, namely:
 - [1] Although claims 1-18 direct to a method for treatment of the amenable skin, the search has been carried out and based on the use of the composition for the manufacturing of a composition for the treatment of the amenable skin.
 - [2] Although claims 19-24 direct to a method of assessing the risk of developing a skin disorder, the search has been carried out and based on the use of the composition for the manufacturing of a composition for assessing the risk of developing a skin disorder.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2016/098822

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