Sytenol® A
The First Natural Alternative to Retinol for Acne-prone Skin
Sytenol® A

Propionibacterium acnes (P. acnes) is a gram-positive normal skin commensal bacterium present in all individuals. Clinically relevant inflammation in acne may be initiated by disruption of the follicular epithelium followed by the spread of bacteria, including Propionibacterium acnes, to the dermis, leading to the development of papules, pustules, and nodulocystic lesions (Zouboulis, Clin Dermatol 22:360–366,2005). P. acnes induces the expression of pro-inflammatory cytokines in various cells involved in cutaneous innate immunity (Ingham et al., J Invest Dermatol 98:895–901, 1992). Although P. acnes is a commensal bacterium of normal skin, it (together with the sebaceous gland) is considered to have an important role in acne development. Several studies have indicated that specific strains of P. acnes bacteria are more commonly associated with acne vulgaris. However, other bacteria (e.g. Staphylococcus and Corynebacterium) can also reside in the follicle and on the surface of the skin.

Acne is a disease of pilosebaceous unit affecting about 80% of teenagers and young adults typically aged 12 to 24 years. Acne is not life threatening; however, it does have a significant psychological impact. Embarrassment, low self-esteem, anxiety, anger, frustration, feelings of depression and social withdrawal may be associated with acne (Baldwin et al., Cutis, 70:133-139, 2002).

Product Information

<table>
<thead>
<tr>
<th>Trade Name</th>
<th>Sytenol® A</th>
</tr>
</thead>
<tbody>
<tr>
<td>INCI Name</td>
<td>Bakuchiol</td>
</tr>
<tr>
<td>Chemical Name</td>
<td>Phenol, 4-[(1E, 3S)-3-ethenyl-3, 7-dimethyl-1, 6-octadienyl]</td>
</tr>
<tr>
<td>CAS #</td>
<td>10309-37-2</td>
</tr>
<tr>
<td>Origin</td>
<td>100% Natural; Extracted and purified from edible seeds</td>
</tr>
<tr>
<td>Appearance</td>
<td>Yellow to yellowish brown liquid</td>
</tr>
<tr>
<td>Purity</td>
<td>95 % min. (Typically, around 99%)</td>
</tr>
<tr>
<td>Solubility/Miscibility</td>
<td>Miscible with a wide-range of hydrophobic emollients</td>
</tr>
<tr>
<td>Stability</td>
<td>Photochemically &amp; hydrolytically stable</td>
</tr>
<tr>
<td>Suggested use level</td>
<td>0.5 to 1%</td>
</tr>
<tr>
<td>Storage</td>
<td>Store in original sealed container at +10 to +30 C; Avoid exposure to light &amp; heat</td>
</tr>
<tr>
<td>Regulatory</td>
<td>Approved for use in all major countries – US, Australia, Europe, Canada, Japan, Korea and many others</td>
</tr>
<tr>
<td>Patents</td>
<td>US 8,529,967; US 8,859,021; Multiple pending US &amp; European patents</td>
</tr>
</tbody>
</table>

What are the Treatment Targets Available for Acne?

No single factor causes acne. Acne is a complex, chronic and common skin disorder. Increased sebum excretion from sebocytes via androgen stimulation & integrity of sebum, bacterial over population, inflammatory cytokines and hyperkeratinization of keratinocytes are the major factors involved in the pathophysiology of acne. Acne research continues to deliver new pieces to the puzzle, and helps us to understand acne pathogenesis and assist the development of new treatment against acne. Therefore, a multi-tasking strategy is needed to improve the conditions of acne-affected skin. Sytenol® A, certainly has some of these key attributes.
Clinically Proven to Mitigate Multiple Signs of Acne

Percent reduction in acne was determined by Global Acne Grading System (Burke et al., British J Dermatol, 111:83-92, 1984). This system takes into account both non-inflammatory and inflammatory lesions. Based on the results, formulations containing 1% Sytenol® A + 2% Salicylic acid showed a nearly 70% reduction in acne lesions and the next best results were with Sytenol® A alone, which reduced acne score by about 57% (Chaudhuri & Marchio, Cosm & Toil, 126:502-510, 2011).

Recently, Polakova et al., reported an 8-week 111 subject study with adapalene (0.1%) vs Bakuchiol + adapalene containing formulations (Polakova et al., Clin Cosmet Investig Dermatol, 8:187–191,2015). Results show statistically significant improvement in all parameters and Bakuchiol certainly improved the treatment outcome of adapalene 0.1% gel in acne subjects. Overall performance, safety and tolerance with Bakuchiol containing formulation was much better than adapalene alone.

Protocol:
- **Subjects** – 15 x 4 with mild (up to 10 comedones) & moderate (10 to 25)
- **Duration of the study** – 6 weeks
- **Frequency of application** – Twice a day
- **Products** – Sytenol® A (1% lotion), Salicylic acid (2% lotion), Sytenol® A + Salicylic acid (1+2% lotion) and Placebo (no active)
- **Assessment of Efficacy** –
  - Acne score at initial day
  - Reduction in comedones after 2, 4 & 6 weeks of treatment
  - Reduction in erythema and pruritus after 2, 4 & 6-weeks of treatment
- **Results** – Sytenol® A alone or in combination with Salicylic acid provide statistically significant improvement in acne-affected skin and is well tolerated.

### Treatment with 1% Sytenol® A Lotion

#### Subject - ID7, Front
- Initial day
- After 6-weeks

#### Subject - ID7, Left
- Initial day
- After 6-weeks

#### Subject - ID9, Front
- Initial day
- After 6-weeks

#### Subject - ID9, Left
- Initial day
- After 6-weeks
Science Behind the Product - Controlling Sebum Production & Its Integrity

Sytenol® A helps maintain the integrity of skin lipids


Squalene production is highly upregulated in acne. As expected, an increase in squalene sets the stage for significantly higher levels of squalene peroxides and diminished vitamin E (Vit.E) in the sebum of acne subjects (Picardo et al., *Dermatoendocrinol*, 1:68-71, 2009). The squalene peroxides confirmed to be highly comedogenic (Chiba et al., *Toxicol Sci*, 25:77-83, 2000), they have recently been reported to set an inflammatory cascade in motion. Specifically, exposure of squalene peroxides to human keratinocyte cells stimulates production of inflammatory cytokines and upregulates lipooxygenase (LOX) activity (Ottaviani et al., *J Invest Dermatol*, 126:2430-2437, 2006). This has been implicated in promoting inflammation in acne even in the absence of *P. acnes*.

When keratinocytes are exposed to *P. acnes* surface proteins, there is an immediate generation of ROS, mainly superoxide. This explains why superoxide dismutase (SOD) and glutathione peroxidase become exhausted due to the burden of oxidative stress, particularly in more severe forms of acne. In papulopustular acne, antioxidant system is reported to be compromised (Basak et al., *J Dermatol*, 28(3):123-127, 2001). It would seem reasonable to assume that clinical interventions with topical agents designated to support the antioxidant defense system and/or direct quenching of radicals generated on the skin surface would be helpful in improving acne-affected skin (Grange et al., *PLoS Pathol*, 5:e1000527, 2009; Whitney et al., *J Drugs Dermatol*, 11(6):742-746, 2012).

Sytenol® A is a broad-spectrum radical and non-radical quencher and has an excellent lipid peroxidation inhibitory activity (Chaudhuri & Bou, *C&T*, 130:63-75, 2015). It is also capable of stimulating antioxidant defense system (Chaudhuri, *EuroCosmetics*, 11-12:20-24, 2015). This dual antioxidant pathways of Sytenol® A can help maintain lipid homeostasis and hence relief to acne-affected skin.

Consequences of squalene peroxidation
**Sytenol® A is a radical & non-radical quencher**

<table>
<thead>
<tr>
<th>Unit</th>
<th>Peroxyl</th>
<th>Hydroxyl</th>
<th>Superoxide</th>
<th>Peroxynitrite</th>
<th>Singlet Oxygen</th>
<th>Lipid Peroxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sytenol® A</td>
<td>15,165</td>
<td>569</td>
<td>204</td>
<td>130</td>
<td>1,325</td>
<td>0.5</td>
</tr>
<tr>
<td>Natural Tocopherol</td>
<td>813</td>
<td>Not detected</td>
<td>Not detected</td>
<td>1</td>
<td>1,110</td>
<td>30</td>
</tr>
</tbody>
</table>

1 µmole Trolox equivalent/g

2 Squalene was used as a substrate for lipid peroxidation inhibitory activity; data is expressed in IC<sub>50</sub> in µg/ml; 60-fold more effective than Tocopherol

**Sytenol® A up-regulates antioxidant defense genes**

**Protocol:**
DNA microarray test using full thickness Epiderm tissue (Mattek)

- Retinol and Sytenol® A were dissolved in DMSO, further diluted in water and applied to full thickness Epiderm tissues
- Incubation for two days
- Selected gene expressions having >± 2-fold change
- Statistical significance: p = ≤0.05

**Roles played by the antioxidant defense genes**

- Glutathione peroxidase (GPX3): Reduces lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water
- Glutathione S-transferases (GSST1 and GSTP1): Regulates the intracellular concentrations of the lipid peroxidation products
- NAD(P)H dehydrogenase (quinone) gene (NQO1): Catalyzes metabolic detoxification of quinones and protects cells against quinone-induced oxidative stress, cytotoxicity, and mutagenicity (Long et al., 60(21):5913-5915, 2000).

**Science Behind the Product - Controlling Bacterial Overpopulation**

The significance of the involvement of *P. acnes* in acne pathogenesis is still controversial, mainly due to the fact that it belongs to the resident microbiota. Recently, metagenomic analysis demonstrated that while the relative abundance of *P. acnes* were similar in healthy and acne-affected skin, the strain population structures were significantly different in the two cohorts. Certain strains were highly associated with acne and other strains were enriched in healthy skin (Fitz-Gibbon et al., J Invest Dermatol, 133(9):2151-2160, 2013).

Overwhelming body of evidence, however, implicates propionibacteria, and *P. acnes* in particular, in inflammatory acne. *P. acnes* is neither a primary pathogen nor a bystander. *P. acnes* plays an active role in determining the nature and extent of the immune response (Mouser et al., J Invest Dermatol, 121:1226-1228, 2003; Lodes et al., Microbiol, 151(12):3667-3681, 2006). Treatments that reduce *P. acnes* numbers lead to clinical improvement of acne (Thiboutot et al., 15:97-109, 1997). Acne-affected skin has also been reported to have higher levels of Staphylococcus and Candida (Nishijima et al., J Dermatol, 27(5):318-323, 2000; Slobodnikova et al., Phytother Res, 18(8):674-676, 2004).

**Sytenol® A has broad-spectrum antibacterial and antifungal property (MIC values in µg/ml)**

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Sytenol® A</th>
<th>Retinol</th>
<th>Benzoyl Peroxide</th>
<th>Salicylic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. acnes</em></td>
<td>1.5</td>
<td>31</td>
<td>50</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1</td>
<td>N/D</td>
<td>15.6</td>
<td>N/D</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>2</td>
<td>N/D</td>
<td>&gt;100</td>
<td>N/D</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>1.5</td>
<td>N/D</td>
<td>N/D</td>
<td>N/D</td>
</tr>
</tbody>
</table>

N/D = Not determined
Science Behind the Product - Inhibiting Inflammation

Emerging data indicate that acne is a primary inflammatory disease, with histological, immunological and clinical evidence suggesting that inflammation occurs at all stages of acne lesion development (Tenghetti, J Clin Aesthet Dermatol, 6(9):27-35, 2013). The immunochemical pathways underlying the initiation and propagation of the inflammation in acne are complex and still being elucidated but shown to involve *P. acnes* (Ottaviani et al., J Invest Dermatol., 127:2430-2437, 2006). However, inflammatory response can occur in the absence of *P. acnes*, in both early and clinically inflammatory lesions, other pathways must exist.

It is well documented that inflammation is critical to all types of acne lesions and is multifactorial, Sytenol® A is expected to provide relief to acne-affected skin (Chaudhuri & Marchio, Cosmet &Toilet, 126(7):502-510, 2011; Chaudhuri, Cosmeceuticals & Active Cosmetics, 3rd edition, Maibach et al, eds, Chapter 1, pp-1-18, 2015) by inhibiting multiple inflammatory genes and enzymes.

**Sytenol® A down-regulates pro-inflammatory genes**

**Protocol**
DNA microarray test using full thickness Epiderm tissue (Mattek)

- Retinol and Sytenol® A were dissolved in DMSO, further diluted in water and applied to full thickness Epiderm tissues
- Incubation for two days
- Selected gene expressions having >± 2-fold change
- Statistical significance: p = ≤0.05

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene Description</th>
<th>Retinol</th>
<th>Sytenol® A</th>
</tr>
</thead>
<tbody>
<tr>
<td>COX1/PTGS1</td>
<td>Cyclooxygenase-1 (Prostaglandin G/H synthase precursor)</td>
<td>-3.4</td>
<td>-3.6</td>
</tr>
<tr>
<td>PLAA</td>
<td>Phospholipase A-2-activating protein</td>
<td>No effect</td>
<td>-7.7</td>
</tr>
<tr>
<td>PLA2G4A</td>
<td>Cytosolic phospholipase A2</td>
<td>-3.1</td>
<td>-2.6</td>
</tr>
<tr>
<td>PTGER2</td>
<td>Prostaglandin E2 receptor EP2 subtype (PGE2)</td>
<td>-2.2</td>
<td>-2.4</td>
</tr>
<tr>
<td>PTGER4</td>
<td>Prostaglandin E2 receptor EP4 subtype (PGE2)</td>
<td>-3.0</td>
<td>-6.1</td>
</tr>
<tr>
<td><em>HPGD/15PGDH</em></td>
<td>Prostaglandin dehydrogenase 1</td>
<td>+4.1</td>
<td>+21.8</td>
</tr>
</tbody>
</table>

* HPGD is a catabolic enzyme controlling the biological activities of prostaglandins by converting them into inactive keto-metabolites

**Sytenol® A inhibits pro-inflammatory enzymes**

Sytenol® A has inhibitory activity against pro-inflammatory enzymes, such as phospholipase A2 (PLA2) and dose-dependently reduce formation of LTB4 and TXB2 (Ferrandiz et al., J Pharm Pharmacol, 48(9):975-980, 1996). Our study showed that Sytenol A is effective in inhibiting COX-1 IC₅₀ 14.7 µg/ml and COX-2 (IC₅₀ 514 µg/ml) activities. Interestingly, at higher dose (50 µg/ml), Retinol boosts COX-2 activity while Sytenol® A reduced the activity by 40% (Chaudhuri & Marchio, Cosmet &Toilet, 126(7):502-510, 2011). Literature also shows that Sytenol® A has lipooxygenase (LOX) inhibitory activity (Ferrandiz et al., J Pharm Pharmacol, 48(9):975-980, 1996).
Science Behind the Product - Correcting Hyperkeratinization

One of the most crucial initial events in the development of acne lesions is hyperkeratinization in the follicular infundibulum and sebaceous duct resulting in microcomedones. Follicular keratinization seems to be triggered by relative deficiency of linoleic acid and peroxides in sebum (Georgel et al., Infect Immune, 73:4512-4521, 2005). Increased 5-α-reductase (DHT) may act on infundibular keratinocytes leading also to abnormal hyperkeratinization (Akamatsu et al., J Invest Dermatol, 99:509-511, 1992).

Sytenol® A is an excellent down-regulator of Type-1 5-α-reductase

Protocol
- Cell – HaCat human keratinocytes
- Incubation time – 48 hrs.
- Quantification – Immunofluorescence technique; Determined spectrophotometrically at 485/518 nm Ex/Em
- Result – At 10 µg/ml, Sytenol® A down regulates 5-α-reductase by about 40% and compares well with retinoic acid

% Down-regulation of 5-α-reductase expression

<table>
<thead>
<tr>
<th>Additives</th>
<th>Rationale</th>
<th>Use Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salicylic Acid</td>
<td>Helps unclog pores to resolve and prevent lesions</td>
<td>2%</td>
</tr>
<tr>
<td>HydraSynol™ IDL</td>
<td>Regulates follicular keratinization, maintains barrier functions, maintains stratum corneum acidity; Review also Synovea® EL brochure</td>
<td>2-4%</td>
</tr>
</tbody>
</table>

Suggested additives for improving effectiveness of Sytenol® A

Key References

3. RK Chaudhuri, B Ou, Bakuchiol to stabilize retinol and polyunsaturated lipids, Cosm & Toil, 130:64-75, 2015
5. RK Chaudhuri & F Marchio, Bakuchiol in the management of acne-affected skin, Cosm & Toil, 126:502-510, 2011
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