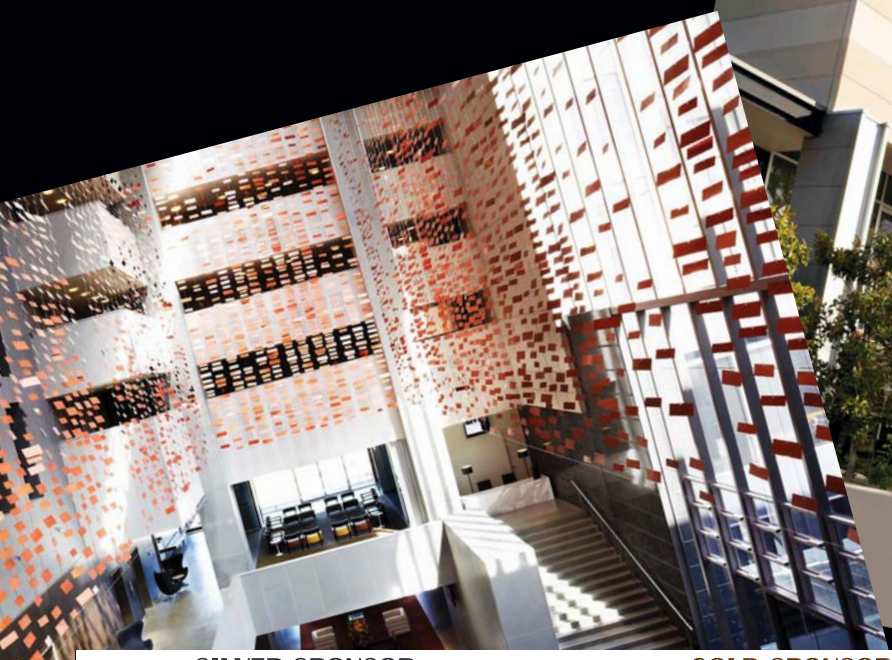
# the science of beauty

Vol 7 No 4

February 2018

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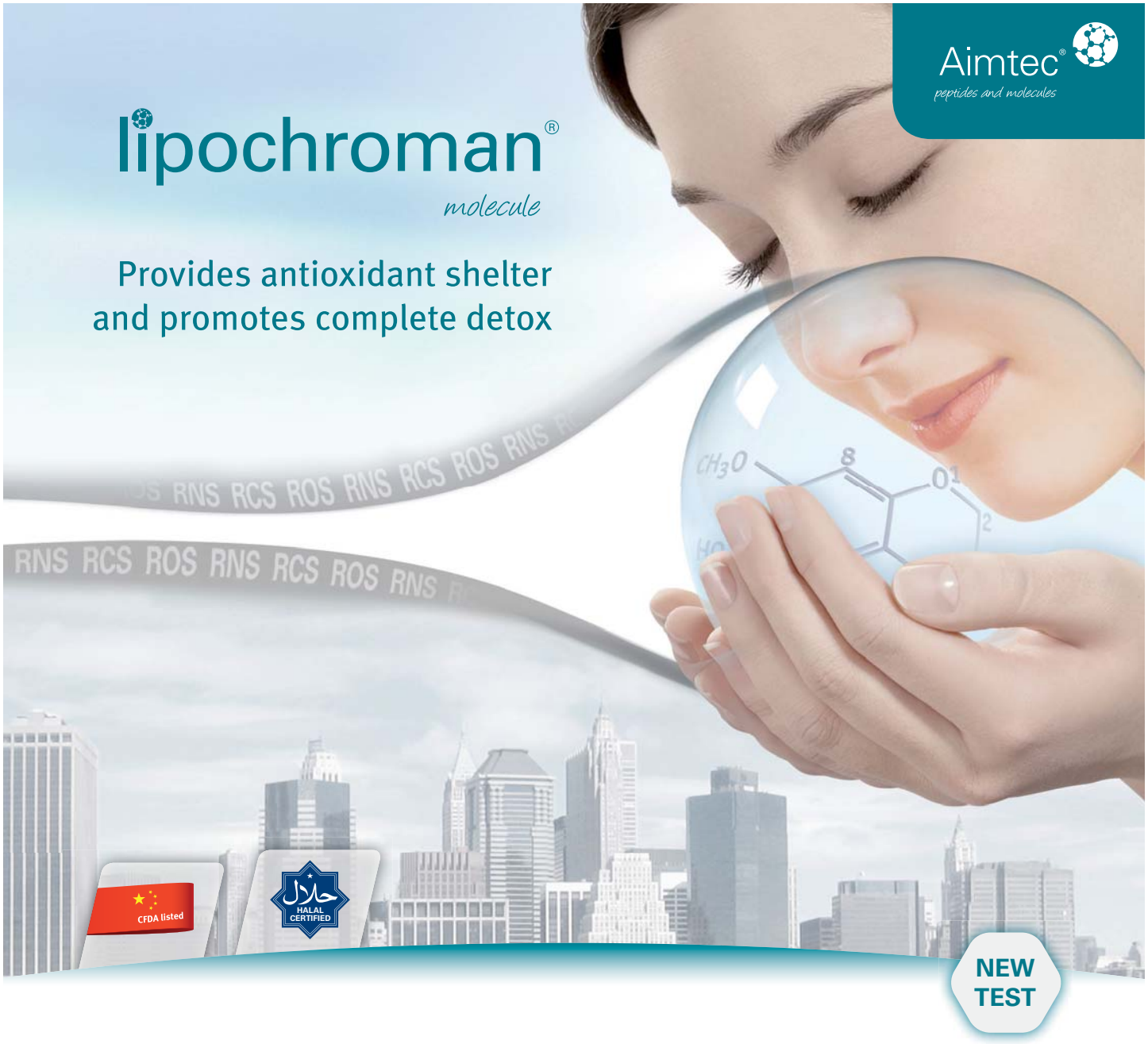
Established 1923

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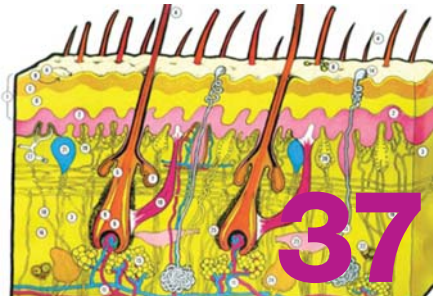


## Advertisers

# Showcase your Innovations in the ASCC Innovation Zone!

Ideal for new material launches and new product offerings to enable visitors to gain inspiration for their next product formulations. There are 20 displays available, allowing **raw material suppliers** to make sure their products will not be missed! Each space is a glass display plinth with top that is 700mm square. Each cabinet is just $395.

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Contact Kate to book your Innovation or Banner opportunity NOW!
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# meet the team...

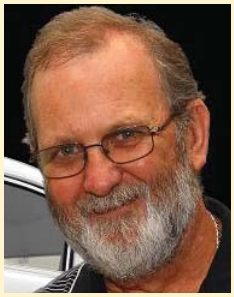
**REBECCA AKHYANI** is a creative perfumer with 15 years experience in the industry. Rebecca has a degree in Industrial Chemistry from UNSW and began her career as a fragrance evaluator before completing perfume school in Grasse, France. Rebecca has worked for a number of fragrance houses in Australia and abroad and is a full member of the British Society of Perfumers. Rebecca also runs perfume classes.

**AUDREY PARATORE** is a professional skin practitioner experienced in many aspects of professional, complimentary and paramedical skin care. She has more than 10 years experience as a Senior Lecturer in Vocational Education and consults for a number of leading skin care companies. Audrey describes herself as a life student of skin science and derives fulfilment in sharing information with other Skin Therapists empowering them to further their careers and bring awareness to the privilege of working hands-on with clients.

**WENDY FREE** has degrees in Science (B.Sc) and Technology Management (M.Tech Mngt) and is a member of a number of industry associations including Australian Society of Microbiologists, Royal Australian Chemical Institute, Association of Therapeutic Goods Consultants and is a Fellow of the Australian Organisation for Quality. With more than 25 years industry experience, Wendy's current roles include APVMA GMP auditioning, contributing to the Cochrane Collaboration and on a day to day basis, Scientific Director Quality Matters Safety Matters Pty Ltd (QMSM) that has over the last decade Wendy has provided expertise to over 400 Australian and International businesses. She specialises in regulatory compliance, commercialisation, troubleshooting and GMP systems, and considers cosmetics amongst the most challenging and enjoyable part of her work.

**PAM STELLEMA** is a business coach (www.salonsavy.com.au) and specialised copywriter (www.salonspacopywriter.com) for salons, spas, clinics and industry suppliers.
Her goal is to help her clients generate greater profits, which she does through her coaching, copywriting, courses, articles and books.
If you'd like to contact Pam, you can phone her on 0431 975 515 or send her an email via either website.

**JOHN STATON** has a background of over 40 years experience in the pharmaceutical and healthcare industries. John is a life member of the ASCC and serves in a number of industry representative roles with ASMI, ACCORD, TGA and Standards. He is the Australian representative to the ISO Committee on Sunscreen Testing-TC 217. (The committee for development of sunscreen standards). John is also in demand as a speaker on the International Conference Circuit.



**RIC WILLIAMS** was educated in Sydney obtaining his Bachelor of Science in Pure and Applied Chemistry from the University of New South Wales (1980) and a Diploma of Environmental Studies from Macquarie University in 1983.
Ric has had 40 years experience in the industry working for many companies and operating his own consultancy business for many years.
He has presented many lectures and workshops at national conferences for the Australian Society of Cosmetic Chemists (ASCC), the Association of Professional Aestheticians of Australia (APAA), Cosmetic and Pharmaceutical Special Interest Group (CAPSIG) and also beauty colleges nation wide.

**TINA ASPRES** has worked as a Pharmacist for almost 20 years in retail, industry and academia as well as being a Cosmetic Chemist. Currently she works in industry and has vast experience in both the pharmaceutical and healthcare arenas. In addition to this she is a casual academic at UTS, School of Health, (Faculty of Pharmacy in Pharmaceutics). Tina has a great interest in clinical research in dermatology and the treatment of skin disease and conditions and is Clinical Trial Coordinator at South West Sydney Dermatology. She is a keen researcher in transdermal drug delivery systems. Tina is a Member of the Pharmaceutical Society of Australia and a Member of the Australian Society of Cosmetic Chemists. She regularly consults pharmaceutical companies in the area of acne, eczema and skincare especially in the area of cosmeceuticals and has devised and written numerous support, training and education material for companies aimed at both professionals and consumers. Tina consults for the Eczema Association Australasia and is on their Integrity Assessment Panel and has worked with Choice Magazine on numerous reports. Tina has presented at the Annual Scientific Meeting of the Australasian College of Dermatologists and has published within the pharmacy and medical literature in the area of sun protection, Vitamin D, skin cancer prevention and eczema as well as co-authoring the book 'All About Kids' Skin – The Essential Guide' published by ABC Books

**STEVE WELSH** is a cosmetic packaging specialist with over 20 years experience across all mediums of packaging. As the director of Weltrade Packaging, Steve leads a team of designers, technicians, printers and supply chain professionals. To ensure the best exposure of your beauty, skincare or cosmetics brand. Steve's philosophy is to design your packaging correctly, right from the start, so you can elevate your brand and move more product. Steve works closely with leaders in the cosmetic industry to ensure that your packaging consistently stands out on the shelves within this highly competitive market.

**GINT SILINS** is a registered patent and trade marks attorney, and a principal of Cullens Patent & Trade Mark Attorneys. He holds a Bachelor of Science degree in chemistry with honours in biochemistry, and a Doctor of Philosophy degree in biochemistry. Gint specialises in protecting branding and innovations largely in the health care, personal care, animal health, food and beverage, biotechnology, industrial chemical, clean energy and agricultural sectors. His practice includes: conducting brand and innovation availability and registrability searches; IP audits; registering patents, trade marks and designs worldwide; enforcing intellectual property rights; resolving IP disputes; and, providing infringement and validity advice.

**MARG SMITH** is the owner of Syndet Works – an Australian company established in 1984 to formulate and produce soap free skincare bars. Syndet has developed an enviable reputation for custom formulated and manufactured skincare that now extend well beyond the origins of the business.

**EMANUELA ELIA** is the Director of Ozderm, which specialises in *in vivo* testing and clinical trials for cosmetic and personal care products. Emanuela Elia has a law degree from Rome and a Master of International Business from the University of Sydney. She had collaborated with Australia's longest serving Contract Research Organisation Datapharm for a few years before setting up a cosmetic and personal care products testing facility in 2009. Emanuela is enthusiastic about improving the quality of cosmetic and personal care products' research in Australia through science.

**CATHERINE CERVASIO** is a business woman with experience in natural personal care, baby skincare, international trade, marketing and branding, spanning two decades. Catherine is most well known for developing Aromababy- the world's first skincare brand to combine the use of natural and organic ingredients with neonatal research, creating a new category in retail in 1994. As the only Australian natural baby skincare brand with registered products in China, she is also sought after as a speaker on accomplishing business in this region. Catherine was a recent winner in CIBE China (Most Popular Natural Brand) and TBPA China (Best Brand Experience) Awards along with winning the HKABA, Export category, for Excellence in Bilateral Trade – China/Hong Kong 2016.

**BELINDA CARLI** is the Director of the Institute of Personal Care Science (www.personalcarescience.com.au), an International Training Organisation providing Certificate and Diplomas via distance education in the formulation, development, brand management and regulatory affairs for personal care and cosmetics.
She is a regular presenter at major International events and her work can be found in many national and International publications and Special Chem formulators site. She is the Official Technical Advisor to the in-cosmetics Group internationally and has written five books on Beginners and Advanced Cosmetic Formulation, Organic and Colour Cosmetic Formulation and Brand Management.

**JAMES GILLARD** is the Principal of Insurance Made Easy whose services include – business insurance, travel insurance and financial services. Insurance Made Easy has a client list of over 2000 businesses from all industries. The relevant major insurance schemes are – Hair and Beauty, Pharmaceutical Companies and Natural Therapists.

# creating your point-of-difference

by Pam Stellema

There are an abundance of salons and spas in the marketplace; I'm sure you won't argue with that statement. Most salons/spas these days have a considerable number of competitors in their own trading zone – if not for all the treatments they provide, then at least for some of them (think hairdressers providing waxing and tinting services or nail bars tapping into manis and pedis).

With so much competition, how can you be sure that your clients will keep coming back to your salon, instead of going to your competitors?

Well, this is when your Point-of-Difference (P.O.D.) comes into play. As a business in a highly competitive field, you must be able to differentiate your salon, and most importantly, your treatments, from the rest; and of course, it must add to the client's enjoyment and results.

When asked, most salon owners tell me their P.O.D. is their exceptional customer service. Now, while great customer service is exceptionally important, this is not a P.O.D. This is simply an expectation on the part of the client.

I have found, from personal experience, that most skincare salons and spas offer pretty great customer service, and because this is usually the case, great customer service is not a viable P.O.D. for most salons or spas.

It needs to be something else; something that other salons and spas in your trading zone simply don't offer to their clients. Something that makes your treatments stand out above your competitors.

While it's fantastic to have a P.O.D. for your salon itself (interesting décor, music, lighting, ambiance), creating a P.O.D. for your treatments is better, and more memorable, for your clients. By creating a truly unique experience, that clients simply can't get elsewhere, your clients have little choice but return to your salon or spa if they want to continue to receive your unique and extraordinary treatments.

## What treatments can have a P.O.D.?

There is not a single treatment you offer that can't be altered just a little to make it better, different and more memorable.

Let me give you a quick and simple example…

A few years back I used to visit a solo-operator for my facials. Her treatment room was at the back of a hair salon (not the most relaxing environment). She was a very caring girl, offered a great facial and was priced fairly for what she provided, *but none of these things, although they are all important, was what ultimately kept me returning.*

Her P.O.D., when delivering facials, was her heated booties. Yup, during each facial (she never missed this step) she

would provide a beautiful foot massage, and pop my feet to gently warmed electric booties, and then continue on with the facial.

Not a big thing you might think, but it made her *stand out* from all the other salons in the area who didn't offer this touch of tootsie luxury. And, for her, the really great part was the fact that it was costing her next to nothing to provide, apart from a little body cream, time and electricity.

This is a great example of having a cost-effective, and therefore sustainable, P.O.D., and ideally, each and every treatment you offer should have one also.

One critical thing to remember however, is that once you've decided on the P.O.D. for each of your treatments, and included them into your treatment protocols, they must be delivered consistently. *I can't stress enough just how important this is.*

Each team member must be trained in the new treatment protocol and must deliver it *each and every time* the treatment is provided. This cannot be a hit and miss activity, as leaving this out will annoy and disappoint once it's been experienced by the client. This, of course, is not what you have set out to do, so never think of it as an optional extra. You must be consistent in your treatment delivery!

## Getting started

To get started, the next step you need to undertake is to brainstorm ideas with your team (if you have one) on how to make each of your treatments unique and outstanding.

> *creating a P.O.D. for your treatments is better, and more memorable, for your clients*

Remember, it's not about adding a great deal of expense or time, but more about finding ways to make each of your treatments more extraordinary and enjoyable for your clients; adding something that they *can't get elsewhere*, and therefore, will keep them returning to you for the experience only your salon offers.

Sometimes, it's even worth putting up the price of the treatment if the P.O.D. adds an additional cost that can't be easily absorbed. Remember, the goal is to create something unique that the client simply can't get elsewhere in your area.

You are only limited by your own creativity and imagination when it comes to deciding on your P.O.D.s. If you're not sure what your competitors are offering, get out and experience some treatments yourself.

If you do this, not only will you appreciate what the treatment feels like from the client's perspective, but you will identify new ideas and possibilities, as well as discover your competitor's weaknesses.

PAM STELLEMA is a business coach (www.salonsavy.com.au) and specialised copywriter (www.salonspacopywriter.com) for salons, spas, clinics and industry suppliers.
Her goal is to help her clients generate greater profits, which she does through her coaching, copywriting, courses, articles and books.
If you'd like to contact Pam, you can phone her on 0431 975 515 or send her an email via either website.

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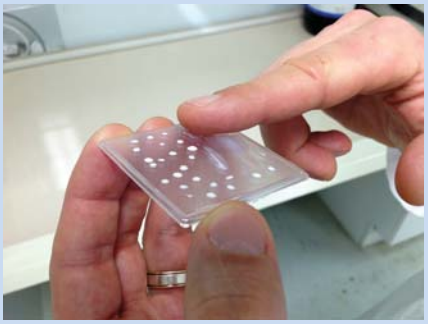
# No. 7 UVAPF BROAD SPECTRUM TEST

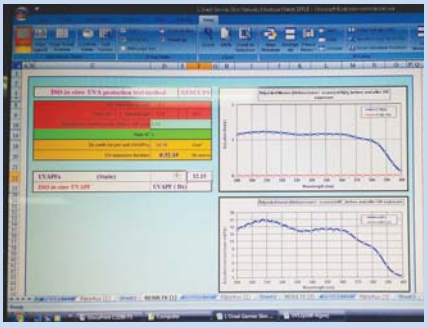
## STEPS



1. Film Preparation

2. UV Light challenge

3. Measurement of UV



4. Calculation of results

**Measurement of UVA Protection In Vitro**

### Supportable Claims

- Complies with ISO 24443
- "Broad Spectrum" – Australia and USA
- UVA Circle – E.U
- Model for PPD – Japan and Korea
- Premature aging Claims

### Principle

The steps of the human UVA tanning test (Persistent Pigment Darkening) can be imitated without the use of test volunteers.

### Steps of the Test

A film of sunscreen is applied onto PMMA plates which imitates skin roughness. Drying is completed. The UV Spectrum is measured. Then the plates are subjected to UV light equivalent to the expected in use performance of the sunscreen. The UV Spectrum is then re-measured. The UVAPF, as well as the Critical Wavelength, can be determined and both broad spectrum and UVA ratio calculated from that data.

### Validation

The instruments for both solar UV light simulation and for spectrum measurement have to be submitted to regular and rigorous calibration

### Reporting

Compliance with all the legislated standards can be certified. The number of markets where this test is accepted in now well over 50.

### For AS/NZS 2604 (2012)

The UVAPF/SPF Ratio must be at least 1/3 and the Critical Wavelength must be at least 370 nm.

This test is...
Compulsory for Primary Sunscreens and Skin Care products
Compulsory for Colour and Lip products with SPF 30 or above.

### For Europe

E.U. requires that this test be performed for sunscreens sold in Europe. It is accepted as the alternative to human testing.

### PPD – Japan & Korea

Both of these markets still require the In vivo test. This instrumental test does correlate and is very useful in order to predict performance before much more expensive human testing.

### References

1.ISO 24443 – Determination of Sunscreen UVA Protection In vitro
2. AS/NZS 2604 (2012) – Sunscreen products – Evaluation and classification

John Staton is a founding Director of Eurofins Dermatest Pty Ltd, Sydney and has been conducting SPF testing and skin efficacy and evaluation studies continuously since 1997.


- Sunscreen Testing
- UVA Testing
- Chemistry
- Stability Testing
- In-Vitro Tests
- Clinical Studies
- Hair Testing
- Consumer Research

Dermatest has recently joined the Eurofins testing group. With over 45 years experience in cosmetics and 27 laboratories worldwide, serving the cosmetic industry and in continuous expansion. This dynamic resource allows us to offer an even broader scope of testing and development services.

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# one more reason why **in-cosmetics Global** is a must-attend event for R&D professionals

The leading personal care ingredients event will focus on R&D at its event in The Netherlands from 17-19 April 2018.

Across the globe, R&D professionals play a hugely important role in the NPD process and, in order to help them innovate for the future, in-cosmetics is launching an exciting new initiative exclusively for cosmetic scientists, chemists and dermatologists at its Global show in April.

For the first time, a series of exclusive 'silent' tours focusing on two highly topical subjects – Biotechnology Actives and The Future of Anti-Ageing – will run throughout the show. Sponsored by Mibelle Group and led by Ms Rouah Al-Wakeel, a leading technical consultant and cosmetic chemist, participants will use wireless headphones to hear information about ingredients and technologies being launched at the show in Amsterdam.

The science and ingredients behind personal care products is pivotal to their success, and the highly curated tour will visit suppliers that have developed some of the most innovative ingredients suppliers in the personal care industry. Participants will get an exclusive demonstration of the products, delivered in a quick, convenient and time-efficient way, allowing them to maximise their time at in-cosmetics Global.

Two groups of 20, twice a day, over two days will be taken around some of the exhibition's most popular areas including the Innovation Zone – a launchpad for more than 100 state-of-the-art ingredients – and the Sensory Bar, where participants will experience a host of new textures and sensations first hand.

Roziani Zulkifli, Exhibition Manager of in-cosmetics Global, commented: "Without the knowledge and expertise of cosmetic scientists and chemists, some of the world's most innovative products would never have made it past their initial tests. We are excited to launch these unique R&D Tours that will help shine a spotlight on new ingredients and help inspire a new wave of products, all in a quick and convenient format."

R&D professionals are invited to book their place on a tour before the show, or at an R&D information point to find out more at the event. For more information, please visit http://www.in-cosmetics.com/.

# Eco-Friendly

**Ecological and Economically friendly.**

For over 60 years, RITA Corporation has specialised in supplying personal care, cosmetic and household markets with a wide range of high quality raw materials and blends.

Consumer demand is increasing for environmentally friendly and natural based materials, as well as ecological and green manufacturing processes. Similarly, there is an increasing emphasis on creating formulations that are energy efficient, whilst reducing manufacturing costs.

RITA Corporation has met these demands by creating products such as Ritafactant SFE, Ritamulse SCG and Ritathix DOE.

Ritafactant SFE is a mild surfactant that can easily be added to the water-phase of cold process production for cosmetic, personal care and household market segments.

Ritafactant SFE is an all-in-one blend and can be used in a wide range of formulas. It is cost competitive, sulphate free, sulphonate free and DEA/MEA free.

Ritamulse SCG incorporates a pre-balanced blend of plant based esters, fatty alcohols, and lactylates into an all-natural emulsifier blend. Ritamulse SCG is sourced responsively (RSPO), completely GMO free and 100% vegan.

Ritathix DOE is a unique surfactant thickener blend, that can be added during any step of the manufacturing process. This eliminates the need for heat and other associative thickeners, all while reducing energy consumption and production costs.

All of these new materials deliver lower costs, streamlined production and more versatile materials that can be used across multiple market segments.

Cold process and natural blends, that offer more "Free From". Free from complexity, free from heating costs and free from formulating headaches.

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Concept Chemical Corporation is ready to support you, along with industry expertise and partnership with RITA Corporation to help meet your supply and new product research needs.

**Contact us on (02) 9498 7600 or sales@conceptchemical.com.au to find out more.**

# 2018 emerging trends for skin care and packaging

by Steve Welsh

Christmas came and went and before you know it, we are into 2018. It already seems so long ago that planning of launch dates were forecast for so many new and exciting products this year.

I recently read a stat that, 33% of consumers will reject a product if they don't like the packaging. In today's growing personal care market, it becomes more important than ever to package well and help the consumer believe in your brand's message.

Recently published in the UK, Bazaar magazine, were the Top 10 Beauty trends for 2018.

**1** Masks, if 2017 was the year of the facial masks then 2018 will be the year of the masks for all other parts of the body. From arms to feet to hair, there will be all sorts of masks formulated using various boutique ingredients to make them appeal to the consumer market. When it comes to packaging we not only deal with the viscosity, but also the seal to make sure they will not dry out. At Weltrade Packaging Solutions, we really like doing it differently. Whether it's the applicator, the shape of jar or the tube that dispenses, we spend a lot of time working through the key requirements so the experience works.

**2** Body care products that are normally associated with the face. In 2018 you can expect to see cleansers, exfoliators, serums, moisturisers with built in SPF factors. Although these are usually associated with facial care products, when it comes to body care, we don't only have to upscale the size of the package, we have to make sure the efficacy of the package is maintained for the shelf life of the formula.

**3** Pro environmentally friendly products and packaging. As consumers become more educated with social media and the pros/cons of various products and their packaging, we are seeing large brands looking to us for their packaging message. Looking to see if there might be a better option for their packaging or wanting our newly released post-consumer resin tubes, (maintaining products and are able to use up to 40% of their weight from recycled plastic) we are always on the lookout for new ways of doing things that is efficient and more environmentally friendly.

**4** Skincare supplements, this is an area

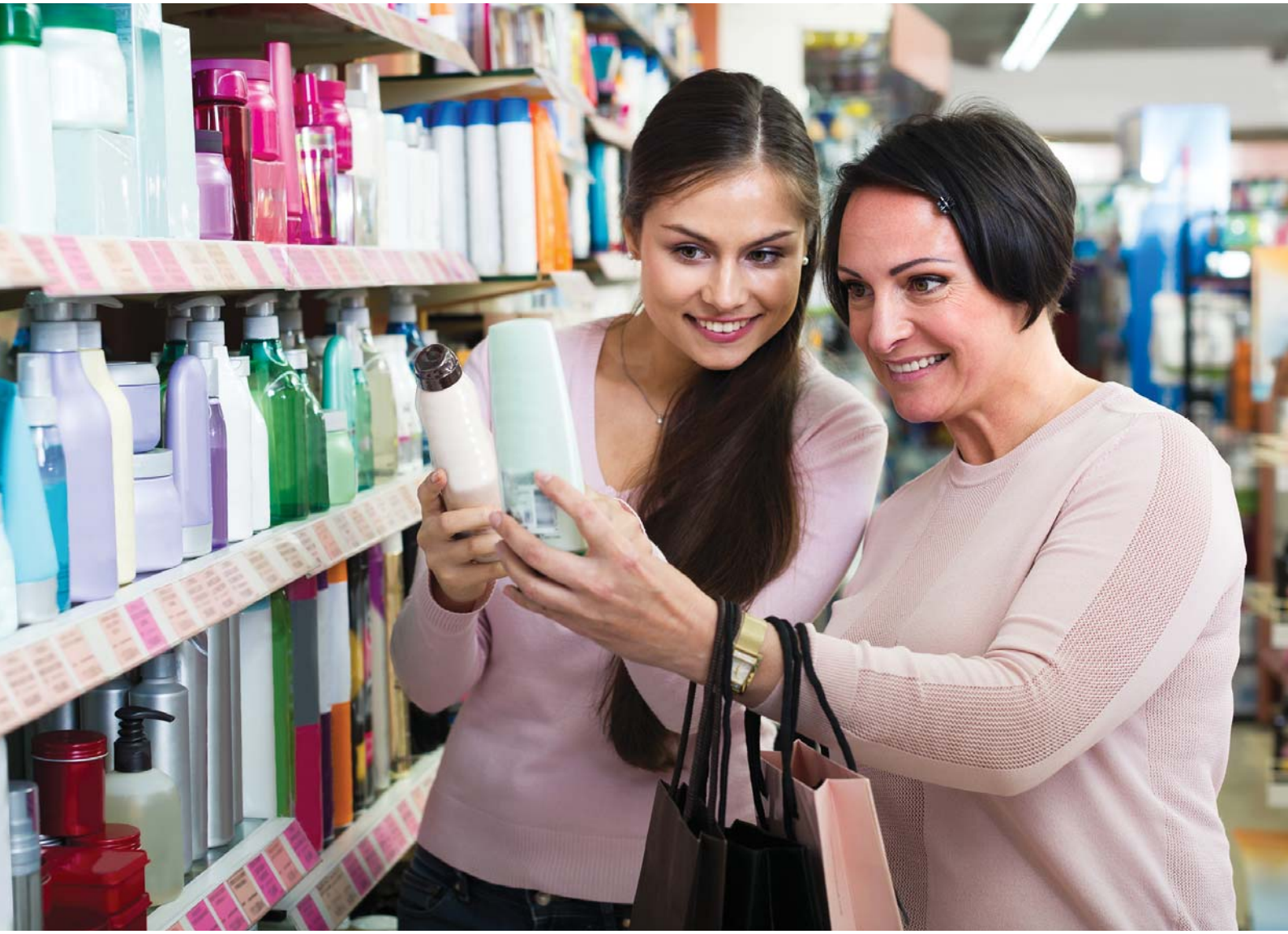
that is rapidly expanding in the beauty industry. Traditionally associated with pharmaceutical and nutraceutical, the beauty effects of supplementing your products is a rising interest for many consumers. Our wellness packaging range has expanded exponentially in the last 12 months and with our new ISO9001:2015 accreditation we expect further growth in this area for us in the beauty industry over the next two years.

**5** Bespoke everything is the new key to really making inroads to markets. Previously we would see brands come in and say, "we are going to do a facial product like brand "x" and we are spending so much on the formulation, although we want the packaging to look great, we just don't have the budget, so show me what stock packaging you have". These days, the more educated brands understand that we can give them something unique, especially in terms of packaging that is really affordable. They then can differentiate their product from others and tailor them to their consumers. As packaging designers, we really love this part of our service to our clients.

Well, that's 1 to 5, of the Top 2018 beauty trends and the packaging discussions we would have with you when discussing your next product launch.

We will cover 6 to 10 in the next issue of Science of Beauty. In the meantime, please pick up the phone or send us an email and we will call you back to have a conversation about your specific needs. We develop our team for your benefit so let's work together to get you the best result.

**STEVE WELSH** is a cosmetic packaging specialist with over 20 years experience across all mediums of packaging. As the director of Weltrade Packaging, Steve leads a team of designers, technicians, printers and supply chain professionals. To ensure the best exposure of your beauty, skincare or cosmetics brand. Steve's philosophy is to design your packaging correctly, right from the start, so you can elevate your brand and move more product. Steve works closely with leaders in the cosmetic industry to ensure that your packaging consistently stands out on the shelves within this highly competitive market.



# Why you should use an insurance broker for all your business insurance needs

by James Gillard

As a new year commences it is important to note that managing your own insurance requires a great deal of expertise. Understandably, you will most likely not have the sufficient skills to identify your insurance needs to select the right cover for your business. Running your business may leave you time-poor and taking on the additional task of organising your own insurance can be a stressful process. It can be a distraction you don't need from the focus of your prime business activities.

## What is the answer?

A qualified Insurance Broker will provide the experience and advice to get it right the first time.

## How do I select my insurance broker?

Find out if they are;

A member of an Association? Members of the National Insurance Brokers Association (NIBA) are the peak body in the insurance industry for insurance Brokers

Are they members of an insurance broker group such as Steadfast? This type of membership provides peace of mind for you knowing the insurance broker has the strength of a large organisation behind them

## Expert knowledge and professional advice

Choosing a Broker who understands your business needs and the industry you trade in is an essential element of you choosing the appropriate Professional. Someone who understands your industry will be able to recommend the most comprehensive and competitive covers. Business Owners should also consider the range of services offered by the Broker and the payment options and processes available to them. Using an Insurance Broker will save you time and money because they can provide you with expert knowledge, advice, and negotiate competitive premiums on your behalf.

## Personalised services

Your insurance Broker should spend time assessing your business risks and investigating the right policy to protect your business.

You should look for:

- Their competitive advantage of the brokerage over others, with attention to their experience in your industry
- How they propose to identify your insurance needs
- Their level of commitment when it comes to your renewals, policy changes and claims service
- Their availability after hours
- Their ability to provide you with the option of dealing with one account executive that you are comfortable with.

## Claims management

The claims process and service you receive is integral to choosing your Broker. Having the right representation

from your Broker relieves much of the anxiety at the time of a claim. Your Broker is your advocate and should be assisting you with the claims process as well as monitoring your claim until finalisation.

## Here to help you

If you are unsure about your current insurance coverage and need a professional advisor to review your policy or risk, and to discuss your own individual circumstances, please contact our friendly team at IME Insurance Brokers.

**Ph 1800 641 260**


# new test on LIPOCHROMAN® molecule

## providing antioxidant shelter and promoting detox

One of the most serious threats for human health nowadays comes from the exposure to a great amount of different chemical substances. The number of chemicals synthesized and used in modern society has grown in the past decades, reaching extremely high levels. These substances are foreign to the organism and are collectively known as xenobiotics.

Modern lifestyle not only involves exposure to thousands of foreign substances but also the production of reactive species within the body itself. These substances, very stable and difficult to eliminate, can accumulate in the body over time causing allergies, inflammation and accelerating external signs of aging.

LIPOCHROMAN® molecule was designed to capture both types of free radicals -RNS and ROS- coming from environmental elements thus protecting cells from damage. Recently, new assays performed on the ingredient have demonstrated a triple protective effect from the reactive species by also capturing RCS, as well as its antioxidative capacity and detox potential.

A new in vitro assay showed how the damage by RCS to cellular proteins was inhibited and skin cells were protected from the genotoxic effect of pollutants.

Bio-inspired antioxidant, LIPOCHROMAN® molecule can assist cells in the elimination of xenobiotics and free radicals coming from both the environment and inner body, thus protecting biomolecules and cellular functions from the damage caused by oxidative stress. A comprehensive solution to fight and prevent premature skin aging.

For more information, please contact Robert McPherson, Account Manager for Australia and New Zeland, at RMcPherson@Lipotec.com or Tel: +61 (02) 9741 5237.

# 2018 Four top trends

by Belinda Carli

As we welcome in a new year, and a more intense focus than ever on R&D at in-cosmetics Global 2018, what should you look out for and what are set to be the hot trends and topics in the industry and at the show? This summary is a guide to what will not only be hot property in the personal care market for 2018, but what to look out for at the show to help guide and speed your personal care developments over the coming year.

## Trend 1: Seeing is believing

Consumer demand for rapid effects in personal care products has continued to increase over recent years; but its not enough to see rapid effects now – consumers want to be convinced of a products efficacy instantly or even before they've used it. How can you do that, you ask? Here are 3 ways companies are achieving this now – we're bound to even more ideas in this space by the end of the year! Try incorporating 1 or more of these into your next developments…

- Incorporate materials a consumer can see, such as encapsulates (biodegradable, of course), small cuttings of plant parts or turn your provide your product as a 'monster' bead or water activated sponge. If they can 'see' the product or active, they're more inclined to believe its efficacy.
- Provide a YouTube demonstration video on how to apply the product or visual results of what it will do for the consumer instantly (in the case of make up or instant effects products) or over time (e.g. time lapse shots over 28 or 56 days through consumer trials) – it not only guides them on best use but visually promises them results beyond marketing words alone.
- Give them a virtual reality: where a consumer can take a picture of their face and watch the virtual results of using your product – think of how on-line ageing simulators work, but in reverse!

When visiting the in-cosmetics Global exhibition, look for materials that will help you give the visual impact either in the packaging (encapsulates or plant parts) or on use!

## Trend 2: It's all about me

Customisation was a big trend of 2017 and its going to get even bigger in 2018. Customisation can be incorporated into new products easily, or an existing range, in one or more ways:

- A 'base' product that consumers can add selected 'active concentrates' to, depending on their skin type needs;
- Products that can be mixed-and-matched from within a brand's range, with accompanying on-line selection form that determines a total 'package' of products depending on their age, skin type and skin concerns;
- Innovative formulation and dual packaging dispensers that can provide partial or complete concentrates or blends of product.

Look for functional materials that provide robust formulation solutions

under varying stressful conditions, so they'll accommodate just about any active concentrate – and then look for those innovative active launches to develop fantastic active concentrates and active products.

## Trend 3: I care about the environment, too

Natural and sustainable ingredients are ongoing favourites of consumers – the demand for these types of materials continues to grow at a steady rate. Look for natural and/or sustainable active and functional materials at the show. Look also for materials that have a 'give back to the community' message or marine actives – 'blue' is the new 'green'.

## Trend 4: Feel good and look better

Products should feel great on application, giving the consumer instant sensory gratification. Not only should products be enjoyable to use, but incorporating epigenetic or neuro-cosmetic actives, for a 'wholistic' approach to their skin care, helps reinforce the consumers' growing need to feel like they're taking control for one aspect of their life and wellbeing. There are also increasing numbers of functional and active materials that help provide 'protection': from UV, pollution, blue light, allergies and other sensitivities – it all adds that 'wellbeing' approach.

When visiting the exhibition, look for materials that give instant sensory pleasures and/or a lasting wellbeing or protection message to fill this growing need.

## Quick highlights

Now for some market segments that are definitely on the up-and-up for 2018:

- Male skin care – we'll see growing diversity and offerings in this area – have you thought about your male consumer yet? Time now to look for materials that will fill this niche as part your product range!
- Microbiome – we've seen materials aimed at balancing the microbiome to solve acne-prone issues; in 2018 we'll see the microbiome do more for a mature market too;
- Mask it – think we're done with mask innovations? Think again! I've already been told about some amazing mask technologies that will have companies rethink their mask development strategies. Attend the 2018 exhibition to see what lies beyond the mask…
- Innovative product forms – materials you know and love – and some you may not know yet – put together in innovative ways to yield the WOW factor and grab your consumers attention. Be at the 2018 in-cosmetics Global exhibition to discover new forms and rethink skin care delivery…

Undoubtedly the worlds biggest gathering of raw material suppliers, the in-cosmetics Global 2018 is an exhibition not to be missed. I look forward to seeing you there as you discover solutions to your formulation challenges and see amazing innovations to take you into a successful 2018!

*Happy formulating!*

Belinda Carli

# Key factors of successful cosmetic trials

by Emanuela Elia

Cosmetic trials are research studies conducted with participants who test cosmetic treatments with the purpose of assessing their efficacy and safety. While laboratory and in vitro studies are important to assess the efficacy and safety of cosmetics, data from clinical trials hold the most relevance to humans. In effect, testing your product in a clinical trial generates scientific evidence for a product's efficacy and safety that is most easily generalisable to the real world.

However, not all cosmetic products tested show positive efficacy outcomes in clinical trials and it is important to understand why this occurs. Proper insight into why a product did not perform as expected is fundamental when determining your next steps, which might include re-formulation of the product, a change in how it is applied, or rethinking how your next experiment should be run in order to maximise the chance of finding a positive outcome.

Any positive (or negative) clinical study outcomes are often due to a large combination of factors. Some of these are directly related to the quality of the test products and their performance, which is beyond the scope of this article. Other factors relate to appropriate clinical trial design and associated study protocol, and these may be issues that could be overlooked due to a lack of understanding or familiarity with statistical testing. If the test product is indeed effective and safe for the skin, the data collected during the clinical trial presents two fundamental characteristics:

## A) The improvement is substantial over placebo

In clinical studies, the most reliable way to assess the efficacy of a treatment is to compare the tested treatment to a placebo control. The average change from baseline to final visit is calculated for each group and then compared together. When treatments are efficacious, treatment results will be higher than the control or placebo, indicating that participants using the treatment experienced a greater change than those using the placebo. Sometimes, the reason why cosmetic studies fail to show a significant change is that the improvements in both treatment and placebo groups are quite similar. Conversely, if the improvement from baseline is substantially greater in the treatment group compared to the control, this result is more likely to be considered statistically significant.

## B) Low variability in the data

When the data collected show high between-subject variability (e.g. the measured effect is positive in some participants and negative in other participants) the effects of the test product are not consistent, and it

becomes harder to confidently say that the average across all participants is representative of the true, underlying value. In this case, although on average the results could be somewhat positive, the spread of data can reveal that either the product's performance or the participants themselves are highly variable, and thus we cannot confidently say that it is significantly different from placebo. Conversely, when the data variability is low, the change after treatment is generally more consistent (e.g. some positive effect in most participants) and therefore displays a better outcome. The lower the variability of data the higher the chances of finding statistical significance in the results.

Figure 1 shows sample data for four possible combinations of effect size and variability. While the points discussed above directly involve the test product and the efficacy data collected throughout the study, below we will touch on some of the elements of the study that may influence the values (point A) and the variability (point B) of the obtained data. All points below are equally important and can ultimately affect the success of a study:

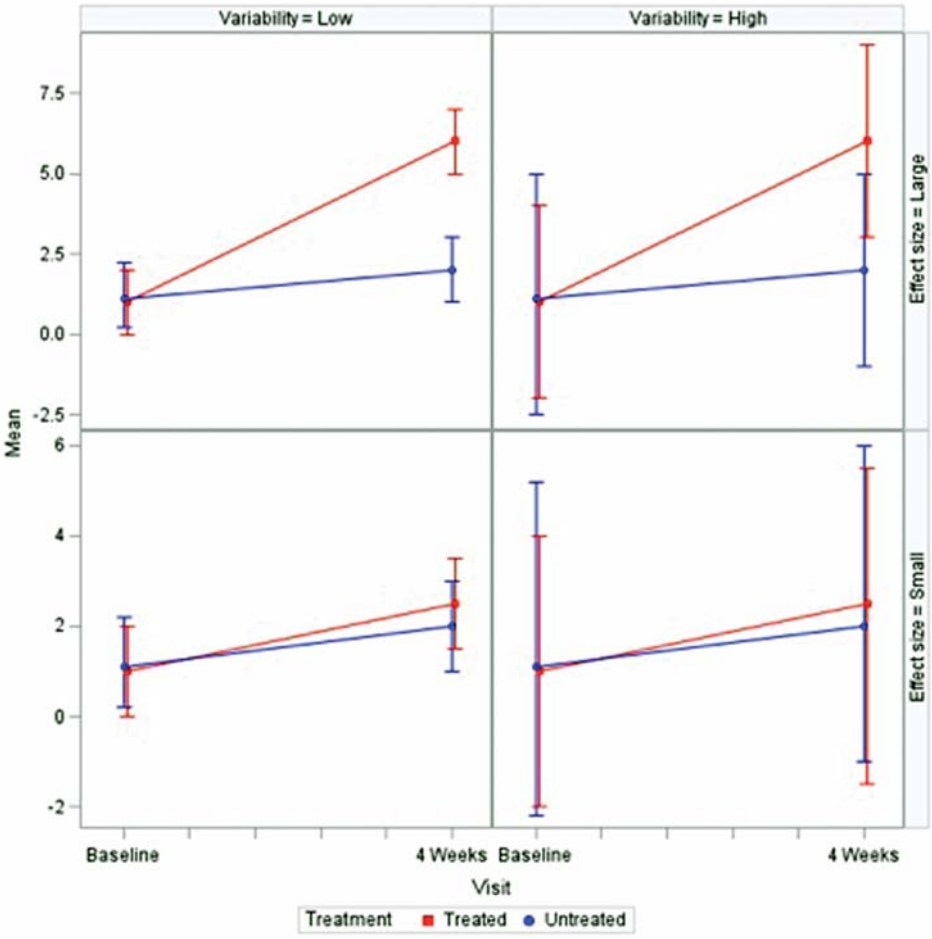



Figure 1. Four possible outcomes of a study according to the effect size and variability of the data.

## 1) Study inclusion/exclusion criteria

Improvement in skin parameters are commonly measured in comparison with baseline (before commencement of the cosmetic treatment) and with a control or placebo. Therefore, to measure a change it is essential that baseline conditions are such that an improvement can occur. Unless the experimental design requires so, there is no point in testing the efficacy of a moisturiser in participants whose skin is already hydrated, for the same reason that you would not enrol young women in an anti-ageing study – participants are unlikely to improve no matter how good your product is!

A study's inclusion and exclusion criteria must take into consideration the target population (i.e. consumers that are likely to use the test product) and exclude those participants that would not benefit from using the test product, as they do not present with the characteristics the test product is intended to improve. Once the target population has been identified, baseline conditions should confirm that improvement with certain skin characteristics is needed, which then allows the product's efficacy to be properly assessed.

## 2) Correct sample size

The size of sample population (i.e. the number of participants in the study) required will depend on the expected size of the effect to be detected, the variability of the data and the power of the study. In general, you will require more participants when aiming to detect a small effect, if you suspect your measurements to be variable, or if you want a greater certainty that the effect you detected is true. For example, the smaller an effect you wish to detect, the larger your study needs to be (all other factors being constant). Conversely, the more effective the product the less study participants you need to find a significant effect.

The power of a study is the probability that it will detect a difference of the magnitude specified if it truly exists. The size of a study should always be large enough to provide a reliable answer to the questions addressed (i.e. have sufficient power). The importance of getting sample size right cannot be overstated: studies with few participants may not allow the detection of differences between groups (It is typical to size studies based on 80% or 90% power). On the other hand, an excess of participants will not only be unnecessary, but costly too. This is why sample size calculation is determined by a statistician based on the primary objective of the study, or by a justification based on statistical and/or methodological expertise (background data, former study, and so on).

## 3) Duration of the study

Duration of the treatment is also key in a study design and needs to be carefully considered to ensure the best

outcome. In some cases, a certain study duration may not seem sufficient to show maximum effect if the effect of the product is a gradual one. In comparison, a longer study could have detected at least an overall trend, which is another method of measuring efficacy (separate from change from baseline to final value). However, a longer study duration does not guarantee better results, it merely provides a greater window in which to detect an effect of the tested product, if that effect indeed exists.

If a trial is successful, the test product group will have a statistically significant greater change from baseline when compared to the control, and this change will have low variability within each test group. When the results of a trial are 'not statistically significant' it means that any observed differences between treatment and control are most likely due to chance. However, it doesn't mean that there truly is no difference, or that the treatment is necessarily ineffective. As discussed above, significant differences in treatment effects in comparison trials might be missed due to other reasons, including incorrect study design in crucial areas such as inclusion/exclusion criteria, sample size and study duration, and should be taken into consideration when assessing your next steps.

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# skin changes in pregnancy

by Tina Aspres

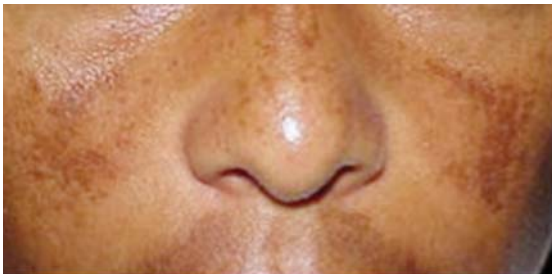
Whilst the saying 'your skin is glowing' is often associated with pregnancy, the reality is that pregnancy is associated with a series of skin changes that will impact a female's life and the skin isn't always 'glowing' and flawless. More than 90% of pregnant females will experience some sort of skin change which may be attributed to an endocrine, vascular, metabolic or immunological change. Most skin manifestations are however, physiological in nature – because of the normal hormonal changes that occur in pregnancy. Other changes that may be seen are in pre-existing skin conditions which may change with pregnancy where one may see an improvement or an exacerbation of a pre-existing skin condition. Occasionally there are rarer pregnancy related skin conditions that may occur during pregnancy or post-partum which require referral for medical opinion and management.

Hormonal changes are attributed to most of the skin changes that are experienced during pregnancy. Nearly all women will experience some degree of hyperpigmentation. There is an increase in serum levels of oestrogen, MSH (melanin stimulating hormone) and progesterone which may cause an increase in pigmentation. Skin conditions caused by normal hormonal changes during pregnancy include a darkening of already pigmented areas, linea nigra and hyperpigmentation.

A darkening of areas that are already pigmented such as freckles, moles, nipples, areolae and labia and even existing scars may be noticed from the first trimester of pregnancy. There is nothing that can be done to prevent this, however, if any change in a mole's shape or appearance is observed, it should be referred to a medical practitioner for review.

Linea nigra is often seen with pregnancy. It refers to a pigmented line that forms down the middle of the abdomen during pregnancy. This usually appears around the fourth or fifth month and there is nothing you can do to prevent it from forming. This form of hyperpigmentation will, however, fade or resolve post-partum and is seldom of any concern.

Melasma/chloasma also referred to as the "mask of pregnancy" on the



other hand is an entirely different problem being the most cosmetically concerning skin condition associated with pregnancy, and can affect up to 70 percent of pregnant women during the second or third trimester or after birth. Skin is usually more sensitive and exposure to sunlight increases the chances of these dark patches on the face. Melasma appears as a symmetrical, butterfly type pattern of darker skin patches on the face – predominantly on the forehead, cheeks, upper lip and chin. Women with tanned or darker skin are most at risk of developing melasma. Once triggered (or worsened) by pregnancy, melasma becomes a chronic problem, and hence lifelong treatment is invariably required throughout life. To try and minimise or prevent melasma, strict sun protection (daily use of broad spectrum at least 30+ sunscreen, wearing of broad brimmed hat and sun avoidance) during and after pregnancy and avoidance of hormonal therapy (such as oral contraceptives) are vital in prevention and treatment of melasma. Treatment options such as hydroquinone, azelaic acid, salicylic acid and tranexamic acid and chemical peels can be considered for the post-partum, non-breast feeding female, but during pregnancy options are limited.

Striae gravidarum (stretch marks) occur in up to 90 percent of pregnant women by the third trimester as the baby grows. They appear as pink to reddish brown or purple streaks on the abdomen. They may also appear on the breasts as the breasts enlarge to prepare for breast feeding, the hips, buttocks and thighs. Striae are more common in younger women, women with larger babies, and in woman with a high body mass index. Whilst there are many products marketed for stretch marks, there is no skin care or treatment regime that can prevent striae developing. A negative family history, optimal body weight, lifelong commitment to exercise, a well-balanced diet and a 'bit of luck' remain the best preventative measures. Once striae develop the initial inflamed purplish appearance will usually fade spontaneously after delivery, although the process may take some 12-18 months. Whilst bland moisturisers and oils may be used during pregnancy, the following treatments can be considered post-partum but none have not been proven to be effective in well controlled clinical trials: laser, topical retinoids, fractionated laser and chemical peels.

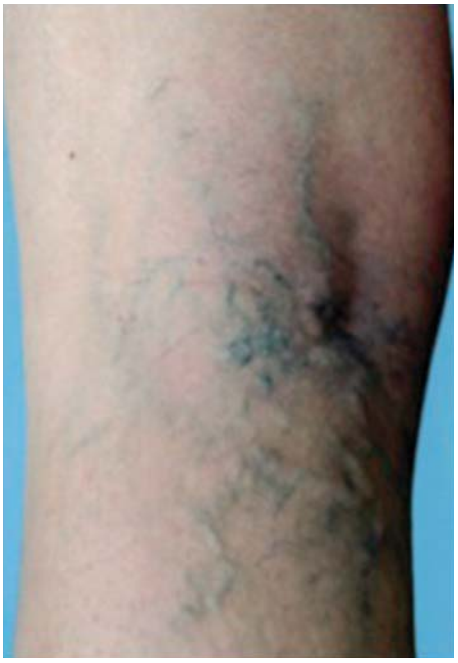
Hormone changes may cause hair and nails to undergo a number of different changes during pregnancy. Women may notice a change in the texture and growth of their hair and nails. Some women report that their hair and nails grow faster and stronger during pregnancy whilst others report that their hair falls out after pregnancy and their nails split, they experience increased brittleness, lifting of the nails and grooves along the nails. Advice is to prevent excessive moisture exposure, damage and irritation to the cuticle area and trauma to the nail plate. This is all that is required as most of these conditions resolve postpartum.

It is normal and most women will experience some hair loss after birth. For some woman, the sudden reduction in oestrogen hormone levels post-partum leads to a marked hair loss from the scalp (telogen effluvium) with a visible reduction in scalp hair density that usually recovers spontaneously within 6-12 months providing any underlying additional trigger such as iron deficiency or thyroid disease are excluded.

With time, hair and nails will often return to what they were prior to pregnancy.

Some women may experience an undesirable hirsutism on the face, limbs and back during pregnancy. Hirsutism associated with pregnancy usually resolves spontaneously post-partum.

Increased progesterone levels and an increase in blood volume can lead to dilatation, fragility and proliferation of blood vessels. Small broken capillaries – spider naevi/veins or spider angiomas (small, reddish blood vessels that branch outward) – occur in about two thirds of lighter skinned females (and 10 percent of darker skin types) primarily appearing on the face, neck, and arms. They usually fade after birth but if they persist post pregnancy, are easily treated with an appropriate vascular laser source.



Varicose veins – enlarged, bluish veins – may appear on the legs, buttock and even vagina during pregnancy as the weight and pressure of the uterus can decrease the blood flow from the lower body. Varicose veins are uncomfortable, may cause the legs to become swollen and may sometimes itchy and painful. Women with a family history of varicose veins may have a propensity to getting varicose veins. In most circumstances, varicose veins are simply more of a cosmetic problem and may go away post-partum. Measures to try and decrease symptoms or prevent them from occurring include avoid standing for long periods of time, wearing of support stockings, walking/exercising regularly, elevating feet when sitting and reducing sodium intake.

Skin may become drier and itchy with pregnancy. Changes may be observed in pre-existing skin conditions. Some skin diseases will improve with pregnancy

whilst others may worsen.

Conditions such as allergic contact dermatitis and hidradenitis suppurativa may improve with pregnancy. Atopic dermatitis and psoriasis may remain stable, improve or worsen during pregnancy. Psoriatic arthritis often worsens with pregnancy. Optimal control of these conditions before pregnancy is recommended.

Pregnancy can cause or exacerbate acne. During pregnancy, hormones may cause an increase in sebaceous gland function, causing the oil glands to produce more oil which in turn can lead to an outbreak of acne. The aim is to control and treat the acne with mild cleansers, oil free cosmetic products and the application of topicals such as benzoyl peroxide (less than 5%), salicylic acid (less than 2%), glycolic acid (not peels) and azelaic acid which are considered safe during pregnancy but it is always best to consult with physician prior to use to ensure there are no contraindications for use. Oral medication to treat the acne such as hormone therapy, isotretinoin, oral tetracyclines and topical retinoids are contraindicated in pregnancy and must be avoided.

It's not all bad skin-wise during pregnancy. With pregnancy, there is an increase in blood volume making the skin appear flushed and plump. In addition, hormones causing sebaceous gland activity increase oil production, making the skin appear smooth and somewhat shiny giving the 'skin glow' appearance. The downside of excess oil production as mentioned above, is acne.

Most skin conditions are benign and usually resolve or can be treated post-partum. When using cosmetic products, it is important to ensure that whatever is being applied to the skin is safe to use during pregnancy. Most cosmetic and over the counter skincare products contain ingredients that are safe to use in pregnancy and when applied to the skin have minimal absorption. Use of mild, soap free cleansers and moisturisers with glycerine, hyaluronic acid and shea butter help maintain skin health. Sunscreen ingredients are also deemed safe and important in skin protection. A few topical products are exceptions and it is probably best to avoid these during pregnancy and nursing eg: products containing hydroquinone, isotretinoin, tretinoin and retinols should be avoided. Essential oils should be used with caution during pregnancy as some may be toxic, contra-indicated or cause adverse events at various stages during pregnancy. Whilst some essential oils are considered safe to use such as lavender, spearmint, ylang ylang, others such as arnica, basil, clary sage, angelica, thyme, cumin, aniseed, citronella, camphor and cinnamon leaf are recommended to be avoided. It is important to check that products are deemed safe before using.

In addition to the common changes the skin undergoes, there are also some uncommon skin conditions that may occur. These conditions are pruritic urticarial papules and plaques of pregnancy (PUPP), prurigo of pregnancy, pemphigoid gestationis and impetigo herpetiformis. These conditions are rarer, vary in gravity, can occur during or after pregnancy and may last several months post-partum and require review by a specialist medical practitioner.

# The Road to China –

## Part Two

by Catherine Cervasio



Product itself plays an important role in China. Despite the huge population and seemingly achievable success purely based on market size, this is not always the case.

Product ingredients, key features and benefits, aesthetics including colour and aroma of finished goods, packaging and price all play a vital part in acceptance by Chinese consumers.

Whilst there is definitely an appeal of 'brand Australia' in China, fierce competition from Europe and USA exists. Chinese consumers seek fancy packaging and less is not always more in the China market, which differs from Australia where we aim to minimise wasteful packaging, reduce our carbon footprint and use recyclable materials, for example. Your brand story is vital in China as it will help form your marketing strategy in the longterm.

Positioning. How you position your brand will impact not only how Chinese consumers perceive your products but may also provide them with insight into your authenticity as a company (or individual). Distributors and consumers alike will check your brand's price and positioning in the Australian market before making a decision to purchase is made.

If you are pitching your brand as mid-high end in China but selling in mass market in Australia or if your suggested retail price in China is considerably higher than in Australia (particularly transparent with ecommerce sites) this inconsistency could be a warning sign for would-be buyers.

Participation. Distributor expectations will vary across China. Some may invest in trade shows on your behalf whilst others may expect you to do this. If you successfully gain product registration and are selling in retail stores, it's likely you will be required to invest in promotional staff, marketing and sampling. You may also be required to train staff directly which has been the case for Aromababy.

My advice for those wishing to export to China is to get on the ground and understand the market for yourself.


First-hand experience and participation in trade shows, trade missions and product showcases can offer valuable insight into how consumers perceive your products and how agents and store buyers view your overall offering. The more experience you have in China, the more you will understand if and how you should adapt your brand or product to suit the Chinese market more specifically.

Persistence. Anyone who has enjoyed any success in China will tell you it doesn't happen overnight. In this market more than any other I have worked in, persistence is key.

In order to sell personal care in Chinese retail stores, for example, there is a rigorous and lengthy registration process to both pass CIQ and ultimately achieve CFDA license approval. This process can take up to 3 years and cost many thousands of dollars per product and at the conclusion of the exercise, products still may not achieve successful registration.

There are various agencies that offer services for handling the product registration process however in my experience, even if you do engage the services of external parties, there is no guarantee of success. Be sure the organization you choose can demonstrate some experience of having successfully gained approval for products in your particular category. Amongst the more heavily regulated are nutritional supplements and baby/mother products.

I recall a presentation by a cosmetic registration agency in China last year, which showed a typical product registration flow chart. It was presented as, what I believed to be, a complex series of slides which demonstrated the multiple steps for applying to register a baby care product. There was also a chart showing the number of successful registrations sitting at around 2000 products in 2006, compared to less than 30 products in 2016. These figures are national and per annum which goes some way to demonstrate the degree of difficulty in getting product onto retail shelves in China.

## Ways to sell to China

Of course there are ways other than retail to sell to China. Alibaba, for example, held its first expo in 2017 and saw strong interest from Australian brands looking to enter the China market via ecommerce.

Whilst having your own brand flagship store on various ecommerce sites may require a sizeable initial investment, there are now numerous ecommerce sites, with Tmall, VIP.com, Kaola.com and JD.com amongst the most widely known.

In addition, there are smaller ecommerce stores on these large platforms which sell Australian made goods. There are stores set up with the primary objective to service 'daigou' (personal shoppers who purchase on behalf of another person and ship the item back to China) and there are even daigou ecommerce sites which make it simple for personal shoppers to purchase with ease and have their goods delivered directly to China without having to visit a physical store.

Whichever way you decide to sell to China, ensure you have researched all the ways available to you, have a long term strategy, build a strong brand story and ideally, have some level of experience in exporting – to a smaller region, for example Singapore or Hong Kong. Be prepared to invest in marketing over and

above what you are currently doing in Australia. Be flexible – you may need to alter packaging, aroma, flavor, product names and even brand names to better suit the Chinese consumer.

Tap into the various government and Chinese business association services on offer and attend events which are focused on export to China. These events are not only great ways to learn from those already working in the China market but also good opportunities for networking.

## Travel to China

Numerous airlines fly direct to major cities in China including Air China and Hainan Airlines however for around the same fare price, because I fly regularly, I prefer to fly Qantas. There's nothing quite like boarding a flight home from China and hearing the Australian accent. Qantas flies direct to major cities including Shanghai and Beijing, however I have also done routing to a variety of other cities along the way which provided a cost-effective way to stop and overnight for meetings and then head off to another city the next day. There are some fantastic deals to China so be sure to keep an eye out.

## Business ettiquette

It may take a little while to get used to the way you need to hail a taxi at the airport, or the fact that there are no seat belts, taxis don't take credit cards and you're likely going to have to haul your own luggage into the boot, but once you know the drill, it all just works.

Here are my top 10 tips for business travel to China.

- Be sure you have the hotel name printed out in Chinese AND English – Google maps won't work in China (nor will your internet search function if you have your browser set to a search engine which is forbidden in China).
- Always carry a small amount of Chinese currency, for the taxi ride to your hotel as a minimum 300RMB. Have an estimate of how much the taxi ride from the airport to your hotel should cost, from someone who knows – I've been charged double (and attempted triple) particularly for late night trips and it's no easy task trying to negotiate in a foreign language. Make sure the meter is turned on.
- Wear comfortable (flat) flat shoes around the airport in particular; some airport gates can be almost a kilometer from the lounges or duty free store areas and you may need to run to catch your flight. Hotels can be located up to an hour car ride from the airports in Shanghai and Beijing, so be sure to use the bathroom at the airport.
- Use the stairs when possible; they are usually less busy than escalators or moving walkways and it may be the only exercise you get when your day is packed full of meetings or you're flying between cities.
- If you can catch a train instead of a plane for domestic travel, do so. I have found it far more reliable and cost effective. Flight delays and cancellations are common for domestic travel so always aim to depart mid afternoon latest if possible. If you're catching one of the last flights out you may well find yourself stranded for the night in the event your flight cannot depart.
- Despite what you hear, there are fewer foreigners than you might think and not many people speak English. I find paying a little extra for 5 star hotels, particularly outside of the major cities, well worth the extra cost for the ease of communication, support, excellent customer service and often late check out (many flights into Melbourne and Sydney depart from Chinese cities late at night).
- Be sure your phone's power bank has markings to show what battery it contains. I can't count the number of portable chargers I've had confiscated at the airport.
- Wifi can be unreliable, even in hotels, so you won't be able to quickly search an address or phone number – always have contact names and addresses printed out in Chinese in case of emergency and particularly when travelling in 2nd and 3rd tier cities
- Social media as we know it is banned in China so if you are managing socials for your business, you will need to schedule posts in advance. Ditto for keeping on contact with friends and family – you won't be able to use Facebook Messenger so use Whatsapp (or Wechat) instead.
- When choosing hotels, why not opt for one of the taller buildings and request a high floor – some of the hotel views are spectacular. It's a great way to take in the enormity of "China". Take a deep breath and enjoy the ride.

# the intensification of fragrances in 2018

by Rebecca Akhyani

How will the year ahead smell? This year's fragrances will be weighing up on the heavy side. Fragrance Evaluator Erica Moore is part of the team at Fragrances of the World, the largest independent guide to fragrance classification. Erica is tasked with classifying hundreds of new fragrances which are launched onto the market every year, often having the opportunity to acquaint herself with a fragrance long before its launch date. She is in a unique position to identify the fragrance trends that we look forward to in 2018. Here she shares with us her insight.

The Oriental, Soft Oriental and Woody Oriental fragrance families take centre stage for men and women. Amber in particular is the note we will see playing a more dominant role than before. The amber note is warm and rich, reminiscent of Ambergris with musky, woody tonalities or the sweet, powdery, earthy character of Labdanum. Dolce & Gabanna's Velvet Amber Skin for women combines Labdanum and leather with vanilla and spices to create a sweet oriental. Also in the spotlight, Calvin Klein Obsessed Eau de Parfum Intense for Men marries vanilla and amber with the earthiness of Guaiac wood to create a woody oriental


Very much in line with this trend the intense note of Oud continues to gain momentum, bringing an explosion of the Woody Oriental family. Oud is derived from agarwood, a plant native to the rainforests of Thailand, Cambodia, Laos and Vietnam. The aroma is a woody with a pungent character, leathery, animalic and smoky. Even Tom Ford have beefed up their renown Oud Wood to deliver Oud Wood Intense. Oud is becoming more familiar amongst feminine perfumes as well, as we see with Aerin's Amber Musk d'Or which combines a delicate floral heart with a dominant Oud note, followed by Amber and soft musk.

Following closely come notes of leather, suede and dry woods which continue to build on this trend. Yves Saint Laurent has launched Sleek Suede, inspired by the leathercraft of Morocco and capturing the softness of the material. The suede note is paired with oud, incense, vanilla and the dry-woody feel of patchouli. A very feminine offering comes in the form of Alaïa Nude by Alaïa Paris. This is an oriental floral in which cedar, musk and leather offset a heart of orange blossom, and the sweetness of tonka bean.

# Brand Management Strategy for Personal Care Businesses

by Gint Silins

In many cases brands ('trade marks') will be the most important assets that a personal care business will ever own (think Olay® $11.8+ billion, Avon® $7.9+ billion and L'Oreal® $7.7+ billion), yet it is often the case that the law surrounding brands is often misunderstood and so brands that are of importance to businesses are not handled properly. Improper handling of brands will often lead to clashes with third parties as well as brand theft, which of course equates to loss of invaluable time, money down the drain and stress. Adopting a sound brand management strategy will help.

This article briefly addresses issues such as trade mark selection, investigation, ownership, use, challenge and protection for both registered and unregistered trade marks.

## Trade Mark Selection

***"Choose a trade mark that is distinctive, capable of distinguishing your goods or services from those of others."***

Any one of the following or any combination of the following can function as a trade mark: letter, word, name, signature, numeral, device, brand, heading, label, ticket, aspect of packaging, shape, colour, sound or scent.

Amongst other things, a good trade mark is one that is capable of distinguishing your goods (products) or services from those of others. For example, a term that describes an attribute of the goods or services is not a good trade mark (eg. SCENTED) and may never be capable of distinguishing your goods or services from those of others. For example, a term that others would need to use in the normal course of trade in respect of their goods or services is not a good trade mark (eg. BEST BUY).

If the world is your proverbial oyster, then at the outset you also need to consider whether your trade mark is likely to be legally, culturally and otherwise acceptable in all other countries of interest. Trade marks that include terms such as AUSTRALIAN, ORGANIC or NATURAL, or certain types of logos, may encounter difficulties in other countries.

## Trade Mark Investigation

***"There is no point choosing a trade mark which you then find is not able to be used commercially."***

An 'availability search' should be undertaken of pending, registered and unregistered trade marks to see whether anybody else has earlier rights to that same trade mark or something confusingly similar to it and could stop you from using it. Perhaps someone else has previously registered the same or similar trade mark? If your investigation reveals that the same trade mark or confusingly similar trade mark is already being used by another person or has been registered by another person for the same or similar goods or services, then rebranding is probably warranted.

***"There is no point choosing a trade mark that is not registrable, that you can never own nor stop others from using."***

Likewise, there is little point choosing a trade mark that is not registrable under the Australian Trade Marks Act

(https://www.legislation.gov.au/Series/C2004A04969) as trade mark ownership and sound protection for that trade mark are unlikely to be available. For this reason, a 'registrability search' should be undertaken to see whether the trade mark is likely to be registrable. Perhaps similar trade marks have been knocked back by IP Australia? Perhaps someone else has applied for registration of the same or similar trade mark?

Where and what to search? Here is a starting point:

1 ATMOSS, being IP Australia's trade marks database for pending and registered Australian trade marks (https://search.ipaustralia.gov.au/trademarks/search/quick).
2 Internet, using different search engines, for trade marks (particularly unregistered trade marks) being used in Australia.
3 ASIC, for registered business and company names (http://asic.gov.au/online-services/search-asics-registers/).
4 Domain names/URLs, as these typically incorporate a trade mark.

If you have a worldwide focus, then similar searches should be carried out in each country of interest to you. Some countries have a freely searchable trade marks database, others don't (eg, USA – https://www.uspto.gov/trademark).

## Ownership

***"Make sure you own the trade mark. If unsure, investigate."***

Generally speaking, in the absence of any agreement to the contrary, the creator of a trade mark/logo is usually the owner of any intellectual property (IP) rights in that trade mark/logo. This means that if you use outside help to design a trade mark/logo for you, then you should ensure that any IP rights in that trade mark/logo (e.g. copyright) are transferred to you such that you own those rights. Normally this would involve putting documentation in place stating that any and all IP rights in the trade mark are assigned to you (normally called a 'deed of assignment'). Not all graphic designers and the like are willing to assign IP rights in the created trade marks to their customers, so you need to be aware of this at the outset and decide whether this is acceptable to you. It makes sense to me that you would want to own the trade mark outright – greater surety and less complications.

***"Make sure that the correct legal entity owns the trade mark."***

Depending on your business structure, which would normally be structured for reasons of taxation and/or asset protection, a trade mark may be owned by a natural person, more than one person, and/or a company or group of companies (or possibly other type of legal entity, eg. an incorporated association).

***"If Entity A owns the trade mark but Entity B is using it, make sure that there is a user agreement between Entities A and B."***

If the owner of the trade mark is not actually using the trade mark, then a written agreement should be put in place between the trade mark owner and actual trade mark user. This is to ensure that any trade mark use is recognised as being for the benefit of the trade mark owner, else trade mark rights or use could be challenged by a third party.

When applying for registration of a trade mark under the Australian Trade Marks Act, here are the usual scenarios:

1 A business owner will apply for registration of the trade mark in his or her personal name because a business name or trading name cannot be a trade mark applicant.
2 A company will apply for registration of the trade mark in its name rather than in the name of its director/s or shareholder/s.
3 A holding company or natural person will apply for registration of the trade mark in its name, even though the trade mark will ultimately be used by a trading company (in which case there should be a written agreement between the parties to ensure that any use of the trade mark is for the benefit of the actual trade mark owner).
4 A company or natural person will apply for registration of the trade mark in their capacity as a trustee for a family trust (ie. Company X as trustee for Y family trust). A family trust as such is not a valid trade mark applicant under the Trade Marks Act.
5 A company or natural person as a place-holder for a corporate entity that has yet to be formed – after which the trade mark application is assigned to the corporate entity.

***"If a trade mark application is not filed in the correct name, it may be defective and unable to be fixed. It may be found invalid if ever contested."***

## Protection and Enforcement

Exclusive rights in a trade mark may be obtained either by use (so-called 'common law' rights) or by registration under the Australian Trade Marks Act.

***"Register each trade mark of commercial importance to you."***

As mentioned above, the most effective way of protecting a trade mark is to register it under the Australian Trade Marks Act 1995. Benefits include: rights can be obtained early, without having to use the trade mark; you can stop others from using the same or confusingly similar trade mark in respect of the same or similar goods and services; a registered trade mark is personal property; a trade mark registration gives Australia-wide rights (unlike common law rights which are restricted to the area in which the trade mark has been used and developed a reputation); the trade mark appears on a searchable database (ATMOSS as well as some international trade mark databases, eg. http://www.wipo.int/branddb/en/) so others can become aware of its existence and may be dissuaded from using the same or similar trade mark; a registered trade mark can be assigned or licensed to a third party; a registration is a defence to an action for infringement of another trade mark (in certain cases); a registration is enforceable in a court of law and remedies include injunctive relief and compensation; and, for enforcement, you need not prove that you have a reputation in the trade mark.

In certain instances rights in unregistered trade marks can be enforced

by way of a 'passing off' action or an action for breach of the Australian Consumer Law or a Fair Trading Act (eg. see http://consumerlaw.gov.au/the-australian-consumer-law/legislation/). Common law rights are usually more difficult and costly to enforce than registered rights. Normally you would need to prove that you have a reputation in the trade mark, else you may not have court-enforceable rights.

Also, if your trade mark is entitled to copyright protection (which is automatic), then enforcement may be possible under the amended Australian Copyright Act 1968 (https://www.legislation.gov.au/Series/C1968A00063).

## Trade Mark Use

***"Use the registered trade mark or lose it."***

If a registered trade mark is not being used (typically for a three-year period), then it is vulnerable to removal from the Australian Trade Marks Register for non-use. If your trade mark has been updated then you should ensure that your current registration still protects the updated version or you should seek further protection for the updated version.

The symbol ® should be used only when the trade mark has been registered, else it is a fineable offence. The registration symbol puts the public on notice that you have registered and enforceable rights in the trade mark. For unregistered trade marks, you should only ever use the notation TM.

## Unregistered Trade Marks

***"Keep evidence of use of each trade mark of importance to your business."***

If for some reason you are not willing or unable to register each trade mark of importance to your business, then you should keep evidence of use of each trade mark from the earliest date possible. Evidence can take the form of advertisements, invoices, promotional materials, tags, packaging materials, products, price lists and photographs showing the trade mark in use. This can be important, usually for the following reasons: 1. A competitor begins using or registers the same trade mark and you need to prove an earlier date of trade mark use so that you can claim ownership and potentially stop that competitor. 2. A competitor sues you for trade mark infringement and you need to prove an earlier date of trade mark use so that you have a defence to infringement and can keep using that trade mark. 3. The trade mark evidence can be used to help secure registration of your trade mark application by way of proving use of the trade mark in the marketplace (i.e. distinctiveness/reputation) or proving that you first used the trade mark before the owner of an earlier cited trade mark – in the latter case IP Australia will be obliged to accept your trade mark application even though the earlier cited trade mark should otherwise block its acceptance and registration.

## Trade Mark Challenges

***"Keep evidence of use of each trade mark of importance to your business in case ownership is challenged, you need to prove earlier use as a defence to infringement, or you need to otherwise prove that your trade mark is being used."***

These comments in quotation marks have been briefly addressed above. Nevertheless, in the case where ownership of the trade mark is challenged, generally speaking, you should be found to be the trade mark owner if you used the trade mark in Australia before the other person first used or applied for registration of the trade mark in Australia.

In the case where you are sued for trade mark infringement, proving that you have earlier use of the trade mark may provide you with a defence to an allegation of trade mark infringement.

In the case where removal of your trade mark registration for non-use is being sought by a third party, evidence of use should put the matter to rest.

Very briefly, trade mark challenges such as trade mark oppositions and applications for trade mark removal for non-use are typically conducted before IP Australia (which is not a court of law). IP Australia's decisions can be appealed to a court of law. Trade mark challenges can also be conducted in a court of law and these are generally more expensive and more onerous than those conducted before IP Australia.

Trade Mark Oppositions – A third party can oppose registration of a trade mark on various grounds, including that: the opposed trade mark is too similar in to an earlier filed pending or registered trade mark that covers the same or similar goods or services; the opposed trade mark is too similar to a trade mark (whether registered or not) that has a reputation in Australia and confusion is likely to occur; the applicant for the opposed trade mark is not the owner of the trade mark under common law (usually because someone else has used an identical or near identical trade mark for the same or similar goods or services in Australia); the opposed trade mark is not distinctive and will not become distinctive; the trade mark application was filed in bad faith; the applicant did not have a genuine intention to use the opposed trade mark at the time they filed the application; and, the use of the opposed trade mark would be contrary to law (eg. if the trade mark is a logo that would infringe the copyright owned by someone else, or if registration of the opposed trade mark would breach some contractual obligation).

A third party can apply for removal or partial removal of a registered trade mark from the Australian Trade Marks Register for non-use on either of these grounds: [1] the applicant for the registered trade mark had no intention at the date of filing to use the trade mark and has not in fact subsequently used the trade mark. (This is a rarely used ground as it is difficult to prove 'a negative'.) [2] The owner or their predecessor/s have not used the registered trade mark in the three year period ending one month before the day when the removal application was filed. (Although this ground is often used, note that applications on this ground can only be made once five years have passed since the filing date of the registered trade mark.)

If an application for removal is not opposed by the trade mark owner, the trade mark will be removed or restricted.

## Brand Management Strategy Summary

1 Check that any trade mark of commercial importance to your business is available for use (eg. company or business name, product name, including any sub-brand that you may possibly initially mistake for a descriptive term of a product).
2 Check that you own each trade mark (if applicable).
3 Check that each trade mark is registrable.
4 File for registration of each trade mark in the correct owner name covering the goods and services of interest to you now and in the foreseeable future.
5 Keep evidence of use of each trade mark (especially in the case of unregistered trade marks).
6 Use the proper trade mark notation, ® or ™.
7 Keep a watch on competitor activities and enforce your rights.
8 If wanting to protect your trade mark internationally, carry out the steps above in each country of interest.
9 Whenever there is a change to your business' branding, revisit the steps listed above.
10 Produce an IP assets document that includes trade marks of importance to your business, such that your business assets can be easily tracked and updated.

Branding, if not managed properly, can lead to all sorts of trouble that can affect your business. Branding, if managed properly, can be extremely valuable business assets. For these reasons it is recommended that your brand management strategy involve a legal advisor.

This article is intended to provide general information only. The contents should not be relied upon as detailed legal advice for any specific case. While every effort has been made to ensure that the contents are correct at the time of publication, please note, the relevant laws and practice are subject to change. Specific advice should be sought from your legal advisor.

GINT SILINS is a registered patent and trade marks attorney, and a principal of Cullens Patent & Trade Mark Attorneys. He holds a Bachelor of Science degree in chemistry with honours in biochemistry, and a Doctor of Philosophy degree in biochemistry. Gint specialises in protecting branding and innovations largely in the health care, personal care, animal health, food and beverage, biotechnology, industrial chemical, clean energy and agricultural sectors. His practice includes: conducting brand and innovation availability and registrability searches; IP audits; registering patents, trade marks and designs worldwide; enforcing intellectual property rights; resolving IP disputes; and, providing infringement and validity advice.

# sunscreen highlights

by John Staton

## Are sunscreens working?

Much has been reported lately (1) (2) regarding the potential for sunscreens to underperform in use and this interpretation impacts on the credibility of both the formulators and the test labs involved, Some SPF testing has been shown to be unreliable and it is evident that there are products in the marketplace where no, little or inaccurate SPF has been reported. Several recent recalls in New Zealand would support this fact (3). Various claims and explanations are made in consumer reports relating to product failure in the marketplace. The most common is the claim that the product has been under-applied. An argument is made along the lines of…

**If sunscreens do not provide the claimed protection due to under application, then why don't more people report being sunburnt?**

ARPANSA has recently added an excellent new tool to the on line real time reporting of UV light. As well as the well recognised UV Index, the data is now being extrapolated to show the predicted and total accumulated dose for major centres each day (4) .The plotting of time versus accumulated dose is displayed in multiples of the Standard Erythemal Dose "SED". This on-line information supports the general maximum of around 70 SED's in zenith

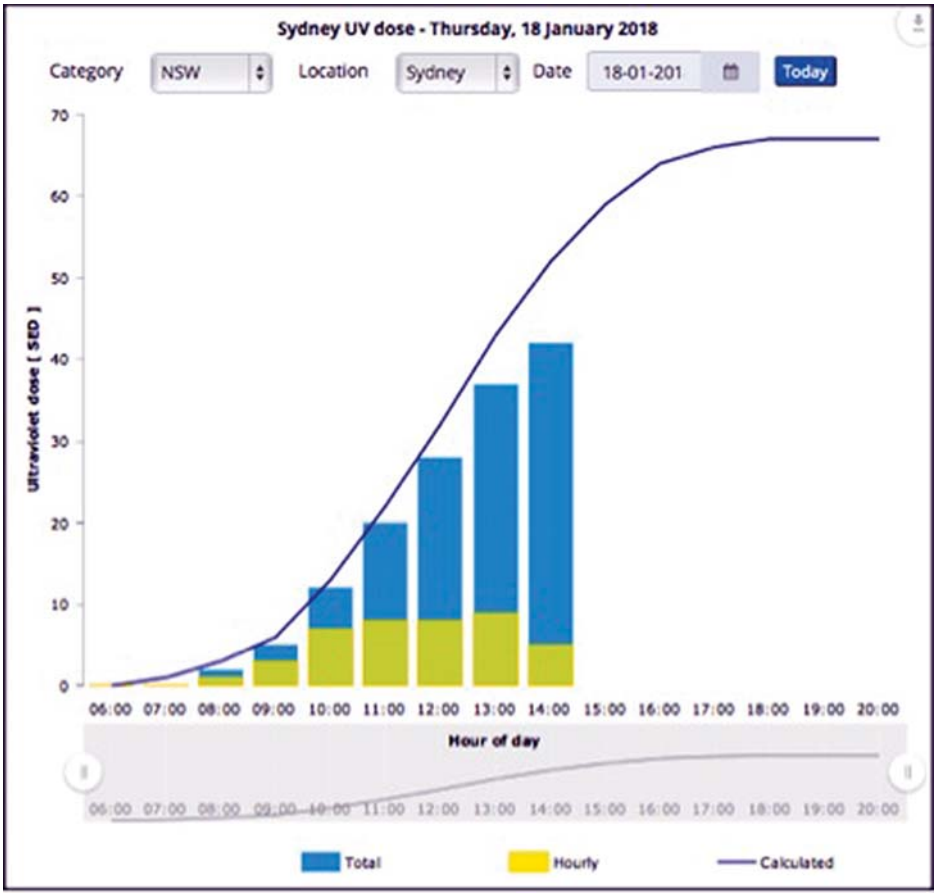



Fig 1. Accumulated UVI Dose Plot – ARPANSA ()

max mid summer in worst case. At Eurofins Dermatest, we looked at their data for 14 years and this figure of 70 SED's appears to be the maximum.

Whilst this term is, at this time, not well recognized, it is important, as it is a **fixed value** independent of skin types and equals 100 Joules per sq.m of erythemally effective energy received onto the unprotected skin. Minimal Erythemal Dose "MED" (the more commonly used term for cosmetic chemistry) is a multiple of the SED. As can be extrapolated from the ARPANSA data, above, it would be rare be exposed to over 70 SED's during a day of maximums. For a Type I skin, 1 MED = 200J/ sq m and dividing by 200/100 J/sq

m the max for ALL DAY i.e 6.00 a.m. to 8:00 p.m. would be 35 MEDs.

Under-application of sunscreen is well recognized. For the thinnest film, the effect of skin roughness comes into play. We showed from our own data that 0.5 mg/sq cm is required to achieve an even film on 6 um roughness PMAA skin imitating plates the valley filling background is valid. However, this effect is ALREADY included in arriving at the SPF when we test on human skin or In vitro.

Some authors claim that 1.25 mg/ sq cm is typical. If 1.25 mg/cm minus 0.5 mg/sq cm 'neutral effect" was the case, then we would see a lot more sunburnt consumers, as 0.75 mg of applied film is 7.5 microns which will typically dry down to 50% of this for a water containing cream/lotion and 20% of this for an 80% volatiles containing spray. Dried film from a spray could then have an theoretical effective film thickness of 7.5 / 5 = only 1.5 um.

I also subscribe to the belief that products are applied by feel, not weight or volume. An older user with rougher skin is likely to compensate to some degree. Similarly, rheology and viscosity will influence the way the film is rubbed out on the skin.

Applying a higher SPF product must compensate to quite a degree. An SPF 50+ has a theoretical at least 50% "safety" margin for under-application, as Beer Lambert should still apply between 2 mg/sq cm, as used in the ISO SPF test and 1.25 mg/ sq cm in use, once 0.5 mg is deducted from both for roughness filling! In this case the effective film thickness is still more than half of what is used in the SPF test. As can be extrapolated from the ARPANSA data, above, it would be rare be exposed to over the equivalent of SPF "35" i.e. 35 MEDs. In effect, SPF 60 should give "all day" protection as long as the product is re-applied as per TGA required label direction.

The major issue remains that a combination of under-application together with over-claimed SPF will provide a greater potential for product failure in use. Both have to be addressed in order to maximise efficacy.

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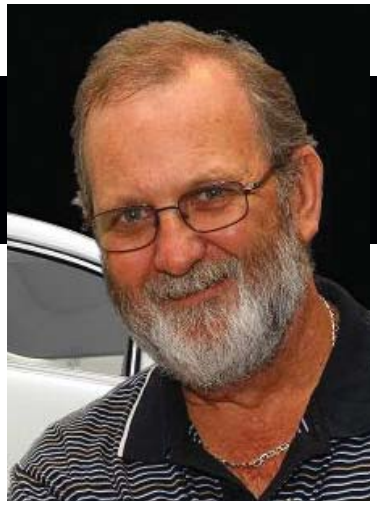
# formulator's forum



## Part 39 –

by Ric Williams

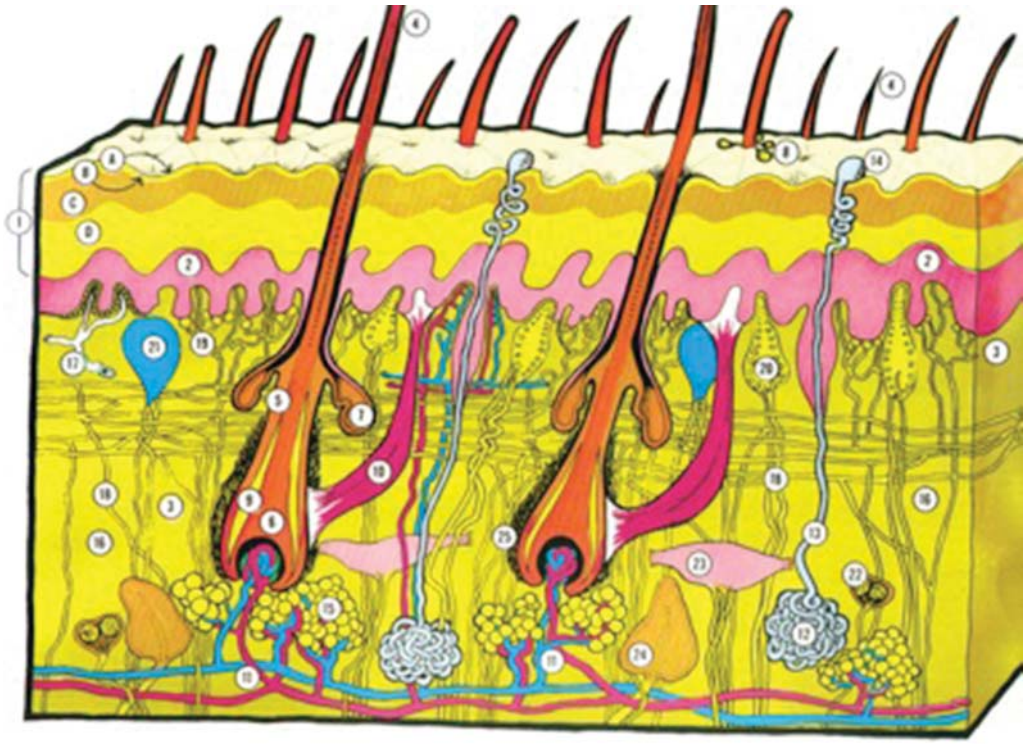
## *Drug Delivery from Cosmetic Emulsions – Part 1*

# Skin

The skin is the largest organ of the body and the one of most interest to Dermatologists, Clinicians, Beauty Therapists and Cosmetic Chemists.

Fig 1

1. STRATUM — *A. Corneum; B. Lucidum; C. Granulosum; D. Spinosum*
2. STRATUM GERMINATIVUM
3. CORIUM
4. HAIRS — *In the center: Medulla; around it: Cuticle*
5. HAIR SHEATH — *outer root*
6. VASCULAR PAPILLA
7. SEBACEOUS GLANDS
8. FAT — *Particles*
9. HAIR SHEATH — *Inner root*
10. ARRECTOR PILI — *Oblique muscle to elevate hair*
11. BLOOD-VESSELS — *Feeding the papilla*
12. SUDORIFEROUS GLANDS — *Sweat*
13. DUCTS — *Sweat gland*
14. DROP OF SWEAT
15. SUBCUTANEOUS FAT CELLS
16. CONNECTIVE TISSUE
17. BRANCH OF LYMPHATIC VESSEL
18. NERVE FIBERS
19. NERVE ENDINGS — *Pain and touch*
20. MEISSNER'S CORPUSCLES — *Touch and pressure*
21. KRAUSE'S END BULBS — *Cold*
22. CORPUSCLES — *Pain and pressure*
23. RUFFINI'S ENDINGS — *Warmth*
24. PACCINIAN CORPUSCLES — *Pressure*
25. HAIR FOLLICLE



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This column is intended not only as an education tool for non-technical people or beginners in our industry, but as a forum for those wishing to enlighten all about recent technology advances and new ideas. I hope experienced scientists will also contribute to this ideal and if you wish to do so please email me at: ric@cosmepeutics.net.au and I will publish your comments.

Skin has many features and functions and one must also realise that the type of skin found will vary depending on which part of the body you look. To exemplify this one only has to look at the inside and outside of the arm. The outside, with more exposure to the sun, is thicker, has more pigmentation, has more hair follicles and is harder (due to a larger quantity of dead "horny layer" cells). Two billion cells which make up the skin are in constant renewal, three hundred million of them being replaced on a daily basis.

Typical functions of the skin are;

a) Protection as a barrier to external chemicals
the most important function in relation to protecting the skin from environmental contaminants. The hard (dead) upper layers acting as a physical barrier while the exuding of sebum provides a chemical barrier that acts to neutralise potential chemical contaminants.

b) Transport of useful chemicals across the skin into the deeper layers, even the blood stream (ie Percutaneous Absorption). Oxygen and Water are typical but the skin also freely allows hormones (steroids) and the fat soluble (A, D, E & K) to enter cell regeneration areas deeper within the skin.

c) Barrier to external factors (sunlight).
Inherent pigment cells offer protection from solar ultraviolet rays – also see Sunscreens

d) Mechanical support.
messy if you don't have it

e) Neurosensory reception.
Pain and touch via many nerve endings that lie just below the Epidermis.
Touch and pressure from the Meissner's Corpuscles located in the upper layers of the Dermis or the other corpuscles (including the Paccinian Corpuscles) located in lower layers of the Dermis.
Temperature sensations via the Krause's End Bulbs that sense cold (located in the upper layers of the Dermis) and Ruffini's Endings that sense warmth (located in the center layers of the Dermis).

f) Endocrinology.
disposal of unwanted chemicals (sweat)

g) Immunology control.
sebum acts as an "antibiotic' to protect the skin from microorganisms and their toxins.

h) Metabolism,
of Keratin, Collagen, Melanin, Lipids and Carbohydrates.
The skin also plays an important role in the synthesis of Vitamin D (useful for the uptake of Calcium), by UV Light, as this can only occur where the UV Light can penetrate, ie in the Dermis.

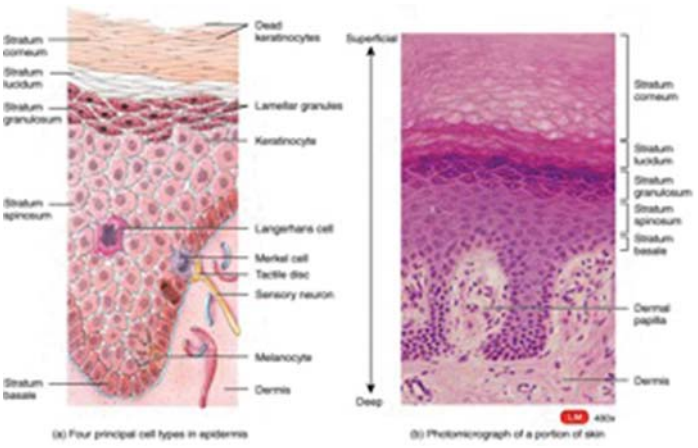
i) Temperature regulation,
the dissipation (radiation) of heat from the vast skin surface (when blood vessels dilate more blood is brought to the surface so that heat can be dissipated), or where the evaporation of surface sweat (from sweat glands), cools the body in hot climates.
The skin surface can also absorb infrared light (from the sun, fire or other heaters) to warm the body in cold climates.
The thickness of skin acts as a physical barrier to rapid heat transfer (in or out).
The circulation of blood maintains the body's temperature in cold climates, because the flow of blood in the veins deep in the subcutaneous tissue protects the Dermis and Hypodermis by maintaining heat in the surrounding tissue.

j) Accentuates aesthetic appeal and beauty.
Apart from colour cosmetics the cosmetic chemist works on skin appearance (wrinkles and defects), skin thickness and softness, moisturisation, and colour.
The Fitzpatrick Classification Scale was developed in 1975 by Harvard Medical School dermatologist, Thomas Fitzpatrick, MD, PhD. This scale classifies a person's complexion and their tolerance of sunlight. It is used by many practitioners to determine how someone will respond or react to facial treatments, and how likely they are to get skin cancer.

| Skin Type | Skin Colour | Characteristics |
|---|---|---|
| I | White; very fair; red or blond hair; blue eyes; freckles | Always burns, never tans |
| II | White; fair; red or blond hair; blue, hazel, or green eyes | Usually burns, tans with difficulty |
| III | Cream white; fair with any eye or hair color; very common | Sometimes mild burn, gradually tans |
| IV | Brown; typical Mediterranean Caucasian skin | Rarely burns, tans with ease |
| V | Dark Brown; mid-eastern skin types | Very rarely burns, tans very easily |
| VI | Black | Never burns, tans very easily |

The skin has a defined structure although the various layers may vary in thickness from area to area.

## The Epidermis

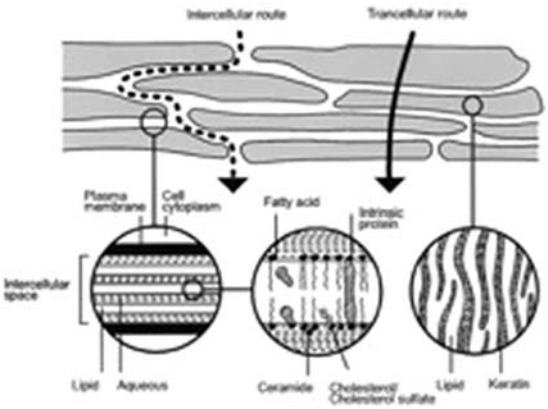


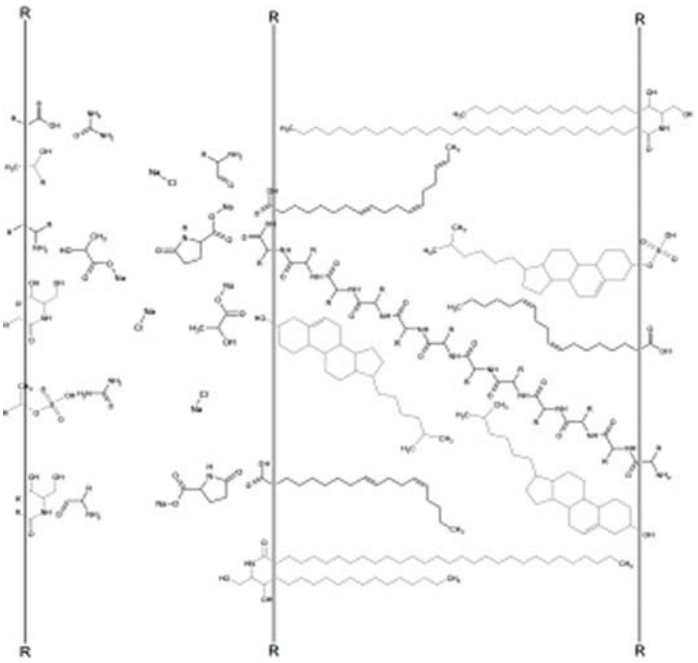

The epidermis is the superficial protective layer at the surface. It varies in thickness from 0.007 mm to 0.12 mm and is composed of stratified squamous epithelium.

The Stratum corneum is the outermost layer, and consists of approximately 25 layers of dead, flat cells composed of overlapping matrix layers of Keratin, with protein deposits.

The fact that keratin is a hard, waterproofing protein this layer acts as the primary (outer) physical protective layer and is the layer that any drug, applied to the surface, must penetrate to have any chance of being absorbed into the body. The outermost cells are continuously lost but are also continually replaced with cells from lower layers (desquamation). It can take from 28 to 55 days from cells to move from the Stratum basale to the surface (ie. across the Epidermis) although this rate will change with age (becomes slower) or illness (eg. in eczema, psoriasis or skin cancers it becomes quicker). I had mentioned earlier that two billion cells which make up the skin are in constant renewal, three hundred million of them being replaced on a daily basis. This desquamation acts to mechanically remove pathogens and other contaminants that are trapped in the outer layer of the Epidermis, but obviously remove beneficial drugs applied to the skins surface as well.

The *intercellular channels* between the layers in the Stratum Corneum are composed of a lamellar structure of lipid layers (nearest the cells) and water layers between (Fig 3,). The water layer is lined with surface active agents that have a polar nature. It is this polar nature that restricts polar chemicals (eg. water soluble vitamins, amino acids and almost all water soluble drugs) from penetrating.



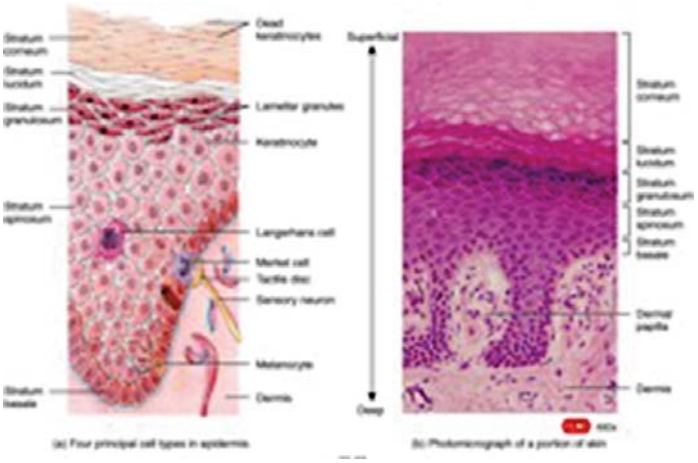




Aqueous layers are like GC Columns

It is lined with surface active agents that have a polar nature, where the ionic groups from fatty acids, ceramides, cholesterol & cholesterol sulfate and proteins, with a polar nature restricts, retards or stops polar chemicals and ionic "drugs (eg. water soluble vitamins, amino acids and almost all water soluble drugs) from passing through the aqueous channels.

Lipid layers are like HPLC Columns

lipophilic "drugs" are retarded or stopped by size exclusion or affinity from the lipophilic portions of the fatty acids, ceramides, cholesterol & cholesterol sulfate and proteins protruding into the lipid layers.




The second layer of the Epidermis is the Stratum lucidium. This layer is more prominent in pressure sensitive areas such as the palms of the hand and soles of the feet. This acts as an extra barrier to physical attack from large sharp objects. The layer consists of rows of clear, flat, dead cells.

The third layer is the Stratum granulosum. This layer (three or four rows of flattened cells) is where the cells begin to die and because of this have a granular appearance, hence its name. Site of formation of keratin complex and lipid synthesis – these lipids form the intracellular cement.

The fourth layer is the Stratum spinosum which contains several stratified layers of living cells, although no new cells are being produced in this layer. This is the site of active protein synthesis generating tonofibrils of keratin that migrate to the granular layer. The cells are not flat but have an irregular shape with sharp edges, giving them a spiny appearance. This layer contains Langerhans cells (nonpigmented granular dendrocytes that are associated with the internal protection of the skin) and activation of these cells is believed to improve the skins protective ability against chemical attack.

The inner (fifth) layer of the Epidermis is the Stratum basale. This is a single layer of cells consisting of keratincytes, melanocytes and tactile cells. Basal keratinocytes divide and migrate to other layers. Melanocytes contain the pigment forming protein, Melanin, which is activated by UV light and gives the skin its ethnic coloured characteristic, a tanned appearance in lighter skin types or such non-uniform features such as freckles and sunspots. To affect skin colour (either to add a tan to fair skin or to lighten ethnic skin colour) this is the layer we must find a pathway to.

## The Dermis

The dermis is the connective layer of the skin (connecting the subcutaneous layer and the Epidermis) comprising collagen and elastin. Because of this the Dermis gives the skin its strength, extensibility, elasticity and "tone". It is also thick in the palms of the hand and soles of the feet while very thin in areas that require extra sensitivity (eyelids, mucous membranes and sexual areas). This is the layer that contains the hair roots, sweat glands, sebaceous glands (sebum production), nerve endings (pain and touch), Meissner's Corpuscles, Paccinian Corpuscles and other corpuscles (pressure and touch), Krause's End Bulbs (cold) and Ruffini's Endings (warmth). Smaller blood vessels also proliferate in the Dermis. The Dermis consists of cellular, fibrous and ground substance components in two layers: Papillary dermis and reticular dermis.

## Facial Muscles

The facial muscles are subcutaneous muscles (just under the skin) that control facial expression and are the prime cause of facial wrinkles. They generally originate on bone, and insert on the skin of the face.

Muscles are contracted when they receive neurotransmitter released from inside a vesicle. The SNARE (SNAp REceptor) complex is essential for this neurotransmitter release at the synapsis (A. Ferrer Montiel et al, The Journal of Biological Chemistry, 1997, 272, 2634-2638). It is a ternary complex formed by the proteins VAMP, Syntaxin and SNAP- 25 (SyNaptosomal Associated Protein). This complex is like a cellular hook which captures vesicles and fuses them with the membrane for the release of neurotransmitter.

Research has shown that by relaxing facial muscles (not tightening the skin), cosmetic peptides, chemicals that mimic the N-terminal end of SNAP-25 which competes with SNAP-25 for a position in the SNARE complex, thereby modulating its formation. If the SNARE complex is slightly destabilized, the vesicle cannot release neurotransmitters efficiently and therefore muscle contraction is attenuated, preventing the formation of lines and wrinkles.

In order to get to these nerve endings we must still aim deeper within the skin as nerve endings affecting facial muscles are in the subcutaneous tissues.

It is apparent hat any drug has a treacherous path to the site of action particularly if this involves blood transport or muscle attenuation. In order to deliver drugs to the blood stream or to affect the sensory organs this is our target area, you can see the difficulties we face.

**Next issue is** *Part 2 of Drug Delivery from Cosmetic Emulsions – Transport of "Drugs" Across the Skin Membrane*

# … going to Europe or …

by Wendy Free

Once we get over the 'big fish' shock and really start getting serious about international compliance it can be very overwhelming. I'm often asked for 'just a simple summary' of what's required for the rest of the world, and I'm very sorry, there just isn't one. It is possible to have one label that matches most of the English speaking world's requirements but it's heavy on text and rules … But let's look at some basics?

## EU (currently applies to UK as well)

1. The ACTUAL requirements[1] are easy read but do require some interpretation.

2. RESPONSIBLE PERSON

The EU RP is the individual or legal entity (ie business) that is "responsible" for the quality and **safety** of individual products. They must ensure that risk assessments etc are properly conducted and 'hold' the data supporting individual product registrations; there legislation describes the obligations and consequences of non-compliance as they pertain to EU RP.

2. Cosmetic Product Notification Portal[2]

Information about products EU RP, formulations, labels and is entered into this secure database by or on behalf of the 'responsible person'. This data can be accessed by the relevant EU authorities. Registered distributors in the EU can check that products are entered and current but cannot access confidential data.

3. The "PIF"

Each product must be supported by a comprehensive Product Information File or PIF; Annex 1 of the legislation[3] prescribed the specific content of the PIF. They are time consuming to prepare[4]; *and the EU RP must be satisfied that the individual preparing the assessment as adequate qualifications, experience etc.*

Start with the full formulation + a representative Certificate of Analysis for every ingredient + the finished product specifications; It's critically important to note that just because an ingredient has an INCI it **DOES NOT MEAN that its 'approved in the EU';** in the EU formulations (not ingredients) are 'approved' via safety assessment … see step 5.

The PIF will also need information about the labels, packaging materials, the product stability and the suitability of the preservative system.

4. GMP – Good Manufacturing Practice.

The recognised standard here is ISO 22716[5]. This standard is a 'stand –alone' one. While a multinational might try to convince you that THEY are the only ones that can certify to this standard, **it's simply not true** … and may not be your best option either.

**ANYONE** can certify to this standard, you can self certify, your dog can certify you … *anyone; so long as the EU RP is satisfied with the certification.*

In Australia there is a small group of independent of "government authorised" GMP auditors[6], and while the government can't issue GMP certificates these auditors[7] are very experienced and may be able to audit and certify you to the satisfaction of your EU RP and your own business standards. Alternatively GMP consultants can be found via the Association of Theraputic Goods Consultants[8], some individuals here may also be well equipped to assist you with Cosmetic GMP establishment and/or certifications. So you really do have a number of options here.

5. Safety Assessment

One of the most fundamental elements of cosmetics is the safety assessment. The legislation requires that an individual who has university level qualifications in a specifically relevant discipline conduct this assessment.

- Start by checking if there are any restrictions on any of the ingredients (preservatives, nano-ingredients and allergens are obvious ones, but also look vitamins A, D and K, that are restricted and/or prohibited in some jurisdictions), and then keep going… most countries have prohibited and restricted lists to check including the EU legislation on the use of substances classified as carcinogenic, mutagenic, or toxic for reproduction (CMR substances), AICS[9], SUSMP[10] etc…
- Then carefully look at impurities *or potential impurities* of "toxicological significance" that might be present in your starting materials[11], (Remember we are looking to find problems NOT to assure ourselves 'its all ok'). In these circumstances the raw, 'natural, and "organic' ingredients can be as bad if not worse than their purified / synthetic counterparts. Colours (colors) also need to be carefully checked.

Once you've looked at the ingredient safety, look at the context for use of the product; will it be used on children, people with physical or intellectual impairments, on shaved skin, around the eyes…could it be swallowed (perhaps because it has a food like aroma or packaging?)…there is plenty of guidance on line if you look for it.

6. Labelling

Most people want to start here, but really it has to be the last step; its not just converting common names to INCI, the allergens in perfumes (and botanical extracts etc) need to be included, as do any relevant warnings or precautions identified during the safety assessment *(mmmm … have you ever wondered about 48 hour antiperspirant protection, why would anyone want you to use less of their product? I wonder … )*

Unless you have stability data to support 30 months shelf life you need to have a 'best before' date on your product as well as a batch number. If you have data supporting at least 30 months you can apply a relevant POA (period after opening) symbol. Your label will also need to carry the name and address of your EU RP.

## Going to the USA?

Unlike Europe where a nominated legal entity is responsible for the product, in the USA; the **MANUFACTURER** is responsible for the product safety … so responsible for all of the above and more …

The regulator is primarily the US FDA[12]; their website provides a lot of information, and while there are only a very few number of substances that are banned in cosmetics, you as the manufacturer are responsible for the product safety, *not the marketing company.*

Fundamentally you must make every effort to make sure that your product is not 'mis-branded' (see flawless beauty recall) or 'adulterated' (see death-wish coffee recall).

Conversely, please be aware these regulations apply to products sold in the USA and not necessarily ones exported from the USA….so if products are *made for export* they may not meet

**From:** **U.S. Food and Drug Administration** fda@go.fda.gov
**Subject:** Flawless Beauty, LLC Issues Voluntary Recall of Unapproved Drugs
**Date:** 24 January 2018 at 9:26 AM
**To:** talktous@qualitymatterssafetymatters.com.au

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FDA U.S. FOOD & DRUG ADMINISTRATION

**Flawless Beauty, LLC Issues Voluntary Recall of Unapproved Drugs**

In accordance with a Consent Decree of Permanent Injunction ordered in the United States District Court for the District of New Jersey, Flawless Beauty, LLC is voluntarily recalling all lots of nineteen different products sold individually or as part of multi-unit kits alleged by the U.S. Food and Drug Administration ("FDA") to be misbranded or unapproved new drugs pursuant to the Federal Food, Drug, and Cosmetic Act. The FDA believes that these drugs present serious public health risks. To date, Flawless Beauty has not received any reports of adverse events related to this recall.

The following products are subject to the recall and were sold and distributed over the Internet to U.S. and foreign customers. The web site of Flawless Beauty is www.flawlessbeautyandskin.com. All glutathione products were sold in multi-vial whitening kits, either alone or in combination with ampules of vitamin C and sterile water. Vials or ampules of vitamin C or sterile water purchased separately or as part of these whitening kits are also recalled:

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From: **U.S. Food and Drug Administration** fda@go.fda.gov
Subject: Death Wish Coffee Co. Announces Recall
Date: 22 September 2017 at 12:59 AM
To: talktous@qualitymatterssafetymatters.com.au

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U.S. FOOD & DRUG ADMINISTRATION

You are subscribed to email messages for the U.S. Food & Drug Administration (FDA).

**Death Wish Coffee Co. Announces Recall of Nitro Cold Brew Cans From Retailers, Online Sales**

Death Wish Coffee Co. ("Death Wish"), the Round Lake, N.Y.-based Coffee producer known for producing the 'World's Strongest Coffee', has initiated a recall its 11-oz Death Wish Nitro Cold Brew cans.

Death Wish in conjunction with an outside Process Authority has determined that the current process could lead to the growth and production of the deadly toxin, botulin, in low acid foods commercialized in reduced oxygen packaging.

Botulism, a potentially fatal form of food poisoning, can cause the following symptoms: general weakness, dizziness, double-vision and trouble with speaking or swallowing. Difficulty in breathing, weakness of other muscles, abdominal distention and constipation may also be common symptoms. People experiencing these problems should seek immediate medical attention.

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the prescribed national requirements; additionally I've seen cosmetics that state that they are only intended for supply by doctors as a 'get-out-of-jail-free' card, they contain drug like substances that are not safe for the general consumer to use without medical advice. While this may be a loop-hole that works in the USA it doesn't fly elsewhere.

In the USA, *once products are on the market* they can be voluntarily registered[13], this might provide you with some means of demonstrating your 'brand' ownership, but perhaps not a lot more.

## Going to ASEAN?

The ASEAN requirements are virtually identical to EU, except

- The local distributors must facilitate to product registration (where required)
- Parfum is called AROMA (or several other names)
- Some animal derived ingredients must be specifically declared on the label
- The product must have a batch number plus a date of manufacturer/or expiry date
- The safety statement is a little different

## Going to New Zealand?

The basic ingredient restrictions are the same as ASEAN/EU but there are also additional requirements for hazardous substances.

So what do they all have in common?

- Everyone requires a complete ingredients list, provided in descending order from 100%, down to 1%, and then in any order. INCI is accepted everywhere and USA and EU co-recognised each others individual variations.
- Everyone requires a weight / volume statement of a particular size, in the EU the height of the volume statement is proportional to the content of the package, in the USA it must be in bold, in the lower 1/3 of the front panel and proportional to the size of the principle display panel (same with Canada….except there it must have a space between the number and the unit of measure ie 500 mL not 500mL), In Australia the height of the statement is proportional to the maximum dimension of the package
- Safety is someone's responsibility

Please feel free to contact me at any time with questions, concerns or requests for subjects for the next article.

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## References

1 http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02009R1223-20160812

2 https://ec.europa.eu/growth/sectors/cosmetics/cpnp_en

3 http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02009R1223-20160812

4 For me they typically take 20 – 40 actual hours, and will comprise over 500 pages of supplementary supporting data.

5 In 2014 I wrote an article about getting started with ISO 22716, please email me if you would like a free copy of this.

6 NB: I am one of these accredited auditors.

7 https://apvma.gov.au/node/26496

8 http://www.atgc.com.au ….also a member

9 https://www.nicnas.gov.au

10 https://www.tga.gov.au/publication/poisons-standard-susmp

11 Substances like heavy metals, formaldehyde, acrylamide, nitrosamines or their precursor amines, dioxin, chloroform, hydroquinone, pesticides, aflatoxins, prions, drug substances (particularly from 'natural' extracts), technical poisons, iodine, boron,…..

12 https://www.fda.gov/Cosmetics/default.htm

13 https://www.fda.gov/Cosmetics/RegistrationProgram/default.htm

# A refreshing new biotechnological ingredient coming from deep international waters to blast the skin with energy and strength

by Silvia Pastor, PhD[1,2], Elaine Ferreira, PhD[2]; Marc Esplugas, PhD2; Víctor San José[2]; Judith García[2]; Patricia Carulla, PhD[2]

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2 LipoTrue Science and Biotechnologies S.L.

## Introduction

Marine environment comprises the major surface area of the planet and is characterized by very special conditions that differ from those found in other habitats. With a wide thermal range (from freezing temperatures in Antarctic waters to about 350°C in deep hydrothermal vents), pressure (1-1000 atmospheres) and nutrients (oligotrophic to eutrophic) to its extensive photic and non-photic zones, this extensive variability has contributed to the great microbial biodiversity observed[1, 2].

With the goal of studying the impact of global change on the marine ecosystem and exploring its biodiversity, particularly in the deep ocean, the Mediterranean Institute of Advanced Studies (IMEDEA) and the Scientific Research Council (CSIC) carried out in 2010, the Malaspina Circumnavigation Expedition where hundreds of samples from international waters and with characteristic environmental conditions were collected. Parameters such as temperature, pressure, salinity and oxygen levels were constantly recorded for samples located down to 4,000 meters' in followed by a full characterization of the strains collection obtained in terms of metagenomics and metabolomics.

LipoTrue S.L. collaborates with these scientific institutions and performs cultures and characterizations of the different microorganisms and their byproducts for the development of new active ingredients with cosmetic applications.

Out of this collection, a bacterial strain (DBSE008), sampled in the Indian Ocean near Reunion Island, was selected. This microorganism is a member of the genus Bacillus with a rod-shaped morphology and was collected at 3,400m depth with existing conditions of 1.5°C and 3.8 µmol/l of oxygen levels.

At LipoTrue's facilities, DBSE008 was grown under very specific and unique fermentation conditions – 10°C and optimal pH and oxygen levels – in order to reproduce as accurately as possible its original living conditions.

The secondary protein/peptide-rich byproducts secreted by this microorganism were characterized for beneficial efficacies on skin cells and explants and have demonstrated amazing energy boosting and firming efficacies.

First of all, the protein-rich extract (INCI: Bacillus Ferment) produced by DBSE008 was analyzed for safety in the main skin cell types and later evaluated on human dermal fibroblasts to determine the specific mechanism of action by gene expression analysis.

Interestingly, genes involved in energy production and protection against oxidative stress (***sirtuin 3***, *SIRT3*) as well as in cell-cell and cell-extracellular matrix (ECM) adhesion (talin 1, TLN1) were upregulated indicating potential effects on cellular energy and redox stability and strengthening between fibroblasts and the ECM.

SIRT3 is the primary mitochondrial

deacetylase[3] and its directly involved in ATP levels increase both in vitro and in vivo[4] being able to maintain mitochondrial energy homeostasis[5] as well as activating the synthesis of dermal proteins[6]. Additionally, SIRT3 increases the activity of antioxidants such as superoxide dismutase 2 (SOD2) and glutathione (GSH) promoting ROS scavenging[7,8] and consequently protecting and prolonging mitochondrial efficiency[9].

On the other hand, TLN1 has been described as one of the key integrin linker proteins which forms a cytoplasmic plaque along other proteins such as vinculin (VCL) participating on mechanosensing events and strengthening cell-cell and cell-matrix adhesions. TLN1 and vinculin associate and bind through integrins, intracellularly to the actin cytoskeleton and extracellularly to the ECM maintaining the mechanical homeostasis in healthy skin[10,11].

The observed changes in the gene expression profile of dermal fibroblasts induced by the extract were later verified by enzymatic activities, specific protein quantifications, energy levels both in vitro and on human skin explants and by means of an undergoing clinical study.

## Materials and methods

### Medium selection and culture conditions

The strain was cultured on different culture media in 125 ml flasks and a marine-based medium showed to be the optimal culture medium. Later on, different culture conditions were tested to determine the best one for growth and production yield.

### Temperature and time selection

To determine the optimum culture temperature and time, several flasks were inoculated at different temperatures ranging from 30°C down to 10°C in order to have this parameter as close as possible to the 1.5°C recorded at the microorganism's collection site. Once the stationary phase was achieved, both growth and protein production from each flask were compared. Assays were performed per triplicate in independent studies to ensure the best conclusive growing conditions.

### Scale-up parameters

Once the medium and the culture conditions at flasks had been optimized, a bioreactor was employed to set the final production parameters. This benchtop fermenter designed to monitor and control parameters such as dissolved oxygen, pH, temperature or stirring was used to define the optimum culture conditions of our production process to generate our active ingredient with a constant yield at any production scale. A constant and reproducible yield at the specific growing parameters was validated after 3 independent fermentations.

### Bacillus Ferment characterization

Cell growth was monitored by measurement of optical density at 600 nm. The supernatant was obtained by cell centrifugation. Once centrifuged, the supernatant was collected and filtered by Polyethersulfone (PES) 0.22 µm in aseptic conditions and freeze-dried. Protein content was quantified by BCA and characterized by SDS-PAGE. Proteins were characterized by silver stain.

### Bacillus Ferment cytotoxicity

Primary human epidermal keratinocytes and primary human dermal fibroblasts were seeded in parallel on 96-well plates for 24h. Later, cells were incubated with 10, 1, 0.1 and 0.01 mg/ml of Bacillus Ferment or with medium only, as a basal control, for additional 24h. Cells viability was evaluated on each cell type using an MTT colorimetric assay which measures metabolic activity of cells by the decrease of the tetrazolium dye MTT to the insoluble formazan, which has a purple color with reading absorbance at 570 nm.

Assay was performed in triplicate for each condition and on three independent experiments. Statistically significant differences between treatments and basal conditions were evaluated by Student's t-test.

### Analysis of gene expression

Primary human dermal fibroblasts were seeded on 6 well-plates in supplemented fibroblasts growth medium and incubated for 24h with either 0.05 mg/ml of Bacillus Ferment or with culture medium only as a control of basal conditions, in duplicate. After incubation, cells from each well and condition were pooled, RNA extracted and samples analyzed for quality using ScreenTape (Agilent) and purity and concentration by Nanodrop. A customized array (Agilent) containing around 600 genes, all involved in skin biological processes was used for analysis. For samples labeling, a Two-Color Microarray-Based Gene Expression Analysis v. 6.5 (Agilent) was used and statistical evaluation was performed with Bioconductor.

### ATP levels evaluation

Normal human dermal fibroblasts (NHDF) were seeded on 96-well plates in fibroblasts growth medium for 48h. Later, culture medium was removed and substituted with a low-glucose medium for 3h. After this period, either fresh normal medium only, as a basal control, or same medium containing Bacillus Ferment at different concentrations (0.01, 0.05, 0.1 and 0.5 mg/ml) was added to the wells and cells incubated for 30 min. After treatments, cells were lysed and intracellular ATP quantified by a luminescence reaction and signal registered by a luminometer.

Assay was performed in six to ten replicates and on three independent experiments. Significant differences between treatments and basal conditions were evaluated by Student's t-test.

### H2O2-induced Reactive Oxygen Species assay

Primary human dermal fibroblasts were seeded in 96 black well-plates in supplemented fibroblasts growth medium. After 24h, cells were incubated with fresh medium only, as a basal control, or with Bacillus Ferment at 0.05, 0.1 and 0.5 mg/ml for 24h. After incubation, cells were loaded

with 2',7'- dichlorodihydrofluorescein diacetate (H2DCFDA), a cell-permeant probe for the detection of reactive oxygen intermediates. Upon cleavage of the acetate groups by oxidation, non-fluorescent H2DCFDA is converted to highly fluorescent 2',7'-dichlorodihydrofluorescein (DCF). After a 45-minutes incubation, oxidative stress was induced by the addition of 100 µM H2O2 for 30 min. Finally, fluorescent signal on each well was measured at Ex485/Em520.

Assay was performed in triplicate and on three independent experiments. Statistically significant differences between treatments and the control of oxidative stress were evaluated by one-way ANOVA followed by Bonferroni's test.

### Elastase activity inhibition

An in tubo evaluation of the efficacy of Bacillus Ferment at inhibiting the activity of the enzyme elastase was performed.

Elastase was incubated either with the compound at 0.005, 0.01, 0.05 and 0.1 mg/ml or with a benchmark control (Phenylmethanesulfonyl, PMSF) known to inhibit elastase activity, in duplicate. Then, the specific enzyme substrate was added and quantification of absorbance at 405 nm was conducted by TECAN Infinite F50 equipment.

Three independent assays were carried out and statistical analysis was performed by a one-way ANOVA test by comparison of test substance vs. benchmark control.

### Procollagen type I quantification

Normal human dermal fibroblasts were seeded in 96-well plates in supplemented fibroblast medium and later incubated either with fresh medium only, as a control of basal conditions, or with Bacillus Ferment at 0.01, 0.05 and 0.1 mg/ml for 48h. After the incubation period, the ex novo synthesis of procollagen type I was evaluated on culture supernatants by a non-competitive ELISA, measuring the absorbance at 450 nm.

Assay was performed in triplicate and on three independent experiments. Statistically significant differences between treatments and the basal control were evaluated by Student-t test.

### Aconitase 2 (ACO2) and vinculin evaluation by immunostaining on skin explants

Skin explants from a 65-year-old Caucasian woman's abdominoplasty were prepared in survival culture medium. Three explants were left untreated in cultured medium for 7 days as the basal condition. On days 0 (T0), T3, T4, T5 and T6, three explants were topically treated with an oil-in-water (o/w) cosmetic formulation only as the placebo condition and another three explants were treated with the same o/w formulation but containing 0.1 mg/ml of Bacillus Ferment. After 7 days (T7) of treatment, half of each explant was frozen at - 80°C and cut into 7-µm-thick sections using a Leica CM 3050 cryostat for immunostaining. Nine frozen sections were used for each condition and for each protein to be evaluated.

For ACO2 immunostaining, a polyclonal anti-mitochondrial ACO2 specific antibody, amplified with a biotin/streptavidin system and revealed by VIP chromogen was used.

For vinculin immunostaining, a monoclonal anti-vinculin specific antibody revealed by the fluorescent marker AF488 (green) was used while cells' nuclei were counterstained using propidium iodide (red).

The different immunostainings were assessed by microscopic observation and image analysis with a Leica DMLB or Olympus BX43 microscope and pictures digitalized with a numeric DP72 Olympus camera. Student-t test analysis was performed to evaluate statistically significant differences between the product and the placebo.

## Results and discussion

DBSE008 produces a protein/peptide-rich derived extract under cold fermentation conditions

SDS-PAGE electrophoresis revealed a characteristic pattern of specific molecular sizes ranging from 20 to 130 KDa (figure 1)

These results indicated that DBSE008 secretes proteins and peptides of different molecular weight when cultured under cold conditions and that these proteins or peptides may have a role on its ability to survive under such harsh conditions.

**Figure 1:** SDS-PAGE electrophoresis gel showing the multiple protein/peptide bands that conform the Bacillus Ferment.

Bacillus Ferment is safe to be used on primary skin cells

Primary human epidermal keratinocytes (HEKa) and primary human dermal fibroblasts (HDFa) from adult skin were incubated during 24h with increasing concentrations of Bacillus Ferment to evaluate the cytotoxicity profile.

Bacillus Ferment showed to be safe on HEKa and HDFa at concentrations below or equal to 10 mg/ml with a decrease in viability of 20% at 10 mg/ml on primary human epidermal keratinocytes and ≤ 11% at 1 mg/ml on primary human dermal fibroblasts, as shown on figure 2.

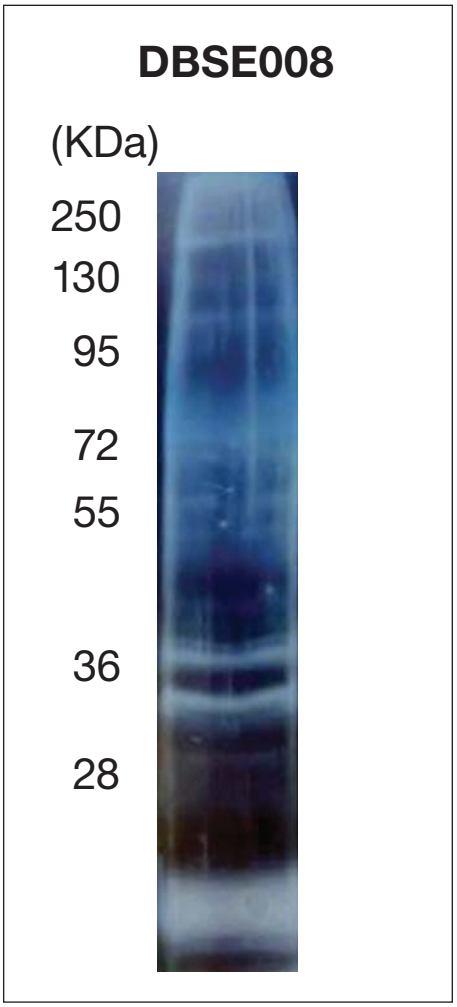
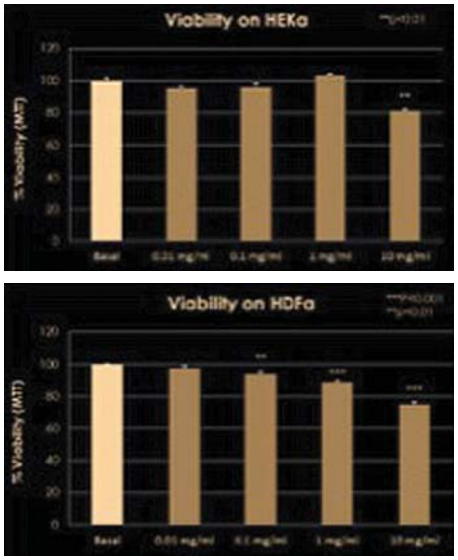


**Figure 2:** Cytotoxicity profile of Bacillus Ferment on primary human skin cells, HEKa and HDFa, after 24h in culture, evaluated by MTT. Conditions were performed in triplicate (n=3) for each concentration and on three independent assays (N=3).

Upregulation of *SIRT3* and *TLN1* by Bacillus Ferment. Two key genes to boost cellular energy and dermal strength.

Primary human dermal fibroblasts were incubated with 0.05 mg/ml of Bacillus Ferment for 24h and gene expression profile was studied by means of a specific microarray. From all genes evaluated, two were significantly increased and showed interesting and potential benefits for the skin, *sirtuin 3* and *talin 1* (figure 3).

*SIRT3* – 10% upregulated – encodes a protein present in mitochondria and is related to ATP production and antioxidant activities. And *TLN1* – 11% upregulated – encodes a cytoplasmic integrin-associated protein located intracellularly with mechanosensing properties.



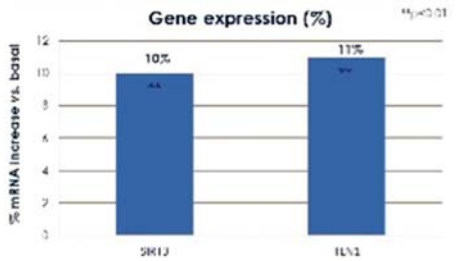




**Figure 3.** Selected upregulated genes (SIRT3 and TLN1) on primary human dermal fibroblasts after a 24h-incubation with 0.05 mg/ml of Bacillus Ferment compared to basal conditions (cells without treatment). Two biological replicates for each condition were used.

The upregulation of these two genes suggested potential effects of Bacillus Ferment. On one side, at increasing cellular energy that could improve cellular metabolism, expression of extracellular proteins, protection from oxidative processes and increase cell longevity12. On another side, potentiate cell-cell adhesion and dermal strength through focal adhesions. All together for a healthful and firming effect on the skin.

The Bacillus Ferment quickly raises ATP levels

Human dermal fibroblasts were incubated for 3h with a low glucose medium to decrease energy availability. After this treatment, cells were incubated with increasing concentrations of Bacillus Ferment for just 30 minutes and ATP levels were measured and compared to cells without any treatment. The energy of the cells increased to a maximum average of 32% on cells incubated with the active ingredient at 0.5 mg/ml compared to basal conditions after glucose decrease (figure 4).

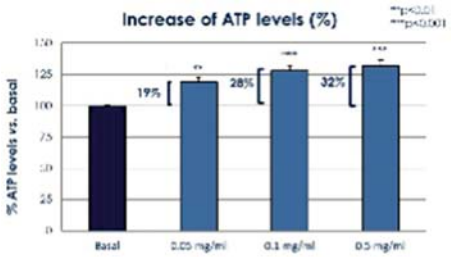



**Figure 4.** Normal human dermal fibroblasts were incubated either with 0.05, 0.1 and 0.5 mg/ml of Bacillus Ferment or with medium only, as a basal control, for 30 min after a glucose decrease period of 3h. Each condition was performed in triplicate (n=3) and 3 independent experiments were carried out (N=3).

These results show that Bacillus Ferment rapidly activates dermal fibroblasts metabolism in a dose-response manner for a potential increase of structural matrix proteins synthesis and cell and cell-matrix strength for a flash and sustained reinforced dermis.

The Bacillus Ferment maintains oxidative homeostasis while boosting energy

After verifying that our marine ingredient was able to significantly increase ATP levels, we wanted to check that the levels of Reactive Oxygen Species (ROS) derived from mitochondrial function during the ATP generation process13 were kept under control.

SIRT3 is known to activate important antioxidant enzymes protecting mitochondrial function and extending cells and tissues lifespan9. Hence, we evaluated ROS levels on human dermal fibroblasts after treatment with Bacillus Ferment at increasing concentrations during 24h (figure 5).

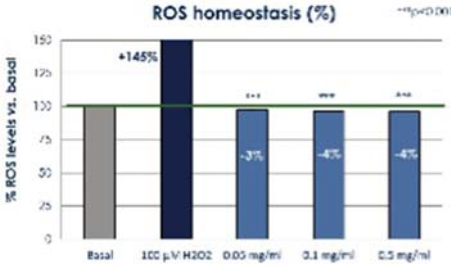



**Figure 5.** Primary human dermal fibroblasts were incubated with either 0.05, 0.1 or 0.5 mg/ml of Bacillus Ferment, 100 µM of H2O2, as a positive control of oxidative stress or with medium only, as a basal control. Each condition was performed in triplicate (n=3) and 3 independent experiments were carried out (N=3).

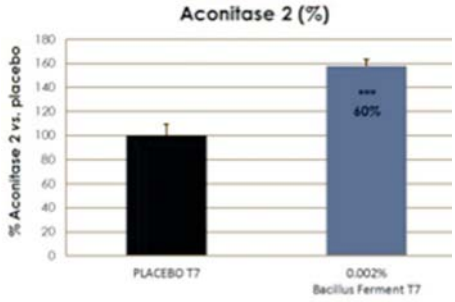
It could be observed that the 24h treatment of dermal fibroblasts with the protein-rich extract did not affect ROS basal levels. On the contrary, 100 µM of H2O2 increased ROS levels by 145% compared to basal conditions.

While a 30-minutes treatment with Bacillus Ferment increased mitochondrial ATP levels up to 32%, this activity was not affecting the redox equilibrium of the cells as measured by ROS levels on cells treated for 24h with the active ingredient. These results suggest that the role of SIRT3 is multifactorial, boosting energy production while keeping endogenous oxidative species levels under control.

Bacillus Ferment is able to upturn the levels of energy on the epidermis

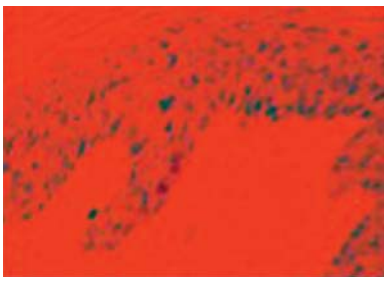
Based on the in vitro results where ATP is significantly and quickly increased, we wanted to measure the levels of aconitase 2, a moonlighting enzyme, able to act on many beneficial aspects on the skin and key in mitochondria for ATP production and longevity[12].

Human skin explants treated either with a placebo formulation or the same formulation containing 0.002% Bacillus Ferment for 7 days and processed as described above, were evaluated for ACO2 expression. Differences between placebo and 0.002% Bacillus Ferment were evaluated (figure 6).




6A

6B



6C

Placebo T7
0.002% Bacillus Ferment T7

**Figure 6.** Levels of ACO2 on skin explants treated with 0.002% Bacillus Ferment in a o/w formulation or without (placebo). Figure 6A shows the average increase of ACO2 on skin explants treated with the active ingredient vs. placebo (n=9). Figure 6B shows a representative image of a skin section treated with placebo. Figure 6C shows a representative image of a skin section treated with the active ingredient. In dark red, ACO2 staining is shown inside cells.

The results showed a good efficacy of the active ingredient at increasing the levels of energy on the epidermis. Mitochondrial ACO2 enzyme participates in energy production breaking down carbohydrates and also moonlights with a secondary role on mitochondrial DNA maintenance by influencing mitochondrial gene expression in response to changes in the cellular environment[14].

With these results, we show that our active ingredient is able to stimulate keratinocytes' metabolism while preserving and protecting the important mitochondria for improved skin health.

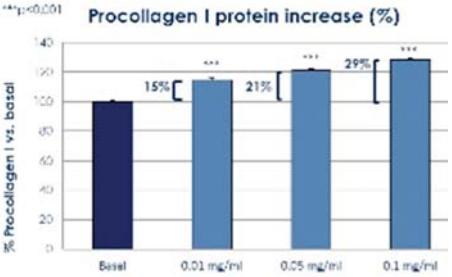
Procollagen type I levels are increased by Bacillus Ferment on human dermal fibroblasts

It is known that several cosmetic benchmarks, like Coenzyme Q10, that also induce an increase in ATP levels and Sirtuin 3 expression[12], have a role in regulating dermal protein production6. Based on this fact, we decided to quantify procollagen type I protein levels.

Human dermal fibroblasts were incubated for 48h with either increasing concentrations of Bacillus Ferment or with culture medium only, as a basal control, and levels of secreted procollagen type I were measured (figure 7).

**Figure 7.** Normal human dermal fibroblasts were cultured with either Bacillus Ferment at 0.01, 0.05 and 0.1 mg/ml or with medium only, as a basal control, for 48h and content of procollagen type I released was measured by ELISA. Each condition was performed in triplicate (n=3) and 3 independent experiments were carried out (N=3).

Our active ingredient showed a good efficacy at increasing the levels of collagen I precursor in a dose-response manner suggesting a redensification of the dermis by boosting procollagen type I. This increase could be mediated by the energizing effect of Bacillus Ferment on the mitochondria with potential efficacies as a firming ingredient in vivo.



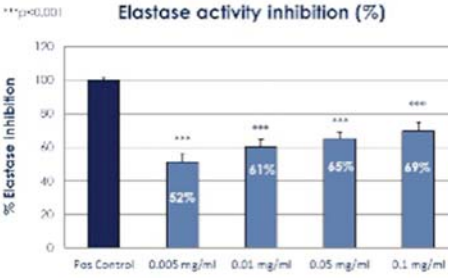

The Bacillus Ferment inhibits elastase activity for elastic fibers protection

After proving the positive effect of energy boosting on dermal proteins synthesis, we also wanted to validate the protective effect of our ingredient against ECM degradation influenced by internal metabolism and external factors. Therefore, we performed an enzymatic test to study the efficacy of Bacillus Ferment at decreasing elastase activity for ECM protection.

An elastase enzyme was incubated either with increasing concentrations of the protein extract or with a positive benchmark control. Later, the enzymatic reaction took place by the addition of a specific substrate.

A dose-response effect was observed with increasing inhibition rates from 52% at 0.005 mg/ml to 69% at 0.1 mg/ml of the active ingredient (figure 8).

**Figure 8.** *In tubo* inhibition of elastase activity by either Bacillus Ferment at 0.005, 0.01, 0.05 and 0.1 mg/ml or with a positive benchmark control. Duplicate (n=2) for each condition and 3 independent assays (N=3) were performed.



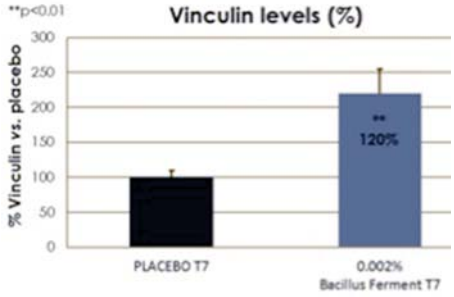

By inhibiting the activity of the ECM degrading enzyme elastase, we show that our ingredient is able to protect elastic fibers from degradation linked

to oxidative stress and inflammatory processes caused from exogenous and endogenous factors.

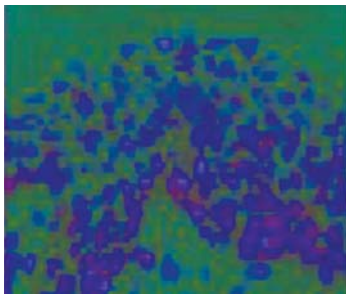
Vinculin, a linker protein with mechanosensing properties, increases its expression after treatment with Bacillus Ferment on skin explants

The observation of an increase in the expression of *TLN1* on dermal fibroblasts led us to evaluate the efficacy of Bacillus Ferment at increasing another associated linker protein on skin, vinculin.

Skin explants treated either with a o/w cosmetic formulation as a placebo or with the same cosmetic formulation containing 0.002% Bacillus Ferment for 7 days and processed as described above, were evaluated for vinculin expression by immunostaining. A significant average increase of 120% was observed on skin sections treated with the active ingredient compared to skin sections treated with the placebo (figure 9).




9A



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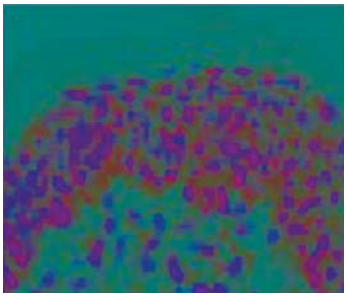

9C

Placebo T7 0.002% Bacillus Ferment T7

**Figure 9.** Vinculin protein expression on human skin explants treated with a cosmetic formulation containing 0.002% Bacillus Ferment or without (placebo). Figure 9A shows the average increase of the active ingredient compared to the placebo (n=9). Figure 9B shows a representative image of one of the skin sections treated with placebo. Figure 9C shows a representative image of one of the skin sections treated with the active ingredient. The green fluorescence marker indicates vinculin protein expression on epidermis and dermis while corresponding nuclei is shown in red after propidium iodide staining.

The histology evaluation indicates the increase of vinculin protein after treatment of skin explants with 0.002% of the active ingredient. The results show that Bacillus Ferment is able to act topically on the skin showing a clear effect at increasing a key linker protein involved in cell-cell adhesion, important for a compacted epidermis and on cell-ECM attachment and strengthening for a tighten and firming effect.

## Conclusions

Out of that large set of microorganisms obtained through collaborations with scientific institutes, a *Bacillus* strain DBSE008, isolated from a 1.5°C water sample collected 3,400m deep near Reunion Island, was optimized in LipoTrue's laboratories for best and most similar culture conditions to the ones found at its collection site.

The freeze-dried Bacillus Ferment obtained was evaluated for safety and transcriptomics on human dermal fibroblasts. Two important genes were significantly upregulated. *Sirtuin 3* is a mitochondrial deacetylase directly involved in the activation of several enzymes of the tricarboxylic acid (TCA) and the electron transport chain where oxidative phosphorylation takes place for the generation of ATP and it is also involved in the synthesis of many antioxidants to guarantee the protection and function of the cellular engine. *Talin 1*, is a cytoplasmic linker protein associated to integrins and strategic for cell-cell adhesion and cell-matrix attachment guaranteeing a full cell communication and dermal strength.

We have demonstrated the ability of Bacillus Ferment to raise ATP levels on cultured fibroblasts and aconitase 2 protein levels on epidermis of skin explants for an improved skin metabolism while keeping oxidative levels under control. We also demonstrated its efficacy at increasing the expression of procollagen type I on fibroblasts and elastase inhibition for dermal reinforcement. Finally, the upregulation of talin 1 was supported by a significant increase of vinculin protein on skin explants. These two proteins provide mechanosensing and invigorating properties to the skin.

An ongoing clinical study is shedding light into the flashing firming effect of this ingredient. 20 Caucasian female subjects aged between 35 and 45 years-old have been included in a single blind study on which a placebo cream is applied on one half of the face and the same cream but containing 2% Bacillus Ferment (0.01%) is applied on the other half, twice a day for 28 days. Instrumental evaluations related to skin firmness and facial reshaping effect as well as skin profilometry around the eyes are undergoing. By now, impressive instant results have been registered after just 30 minutes of treatment with a significant 3% firmness improvement and an average significant decrease of 1.44 mm in length from a fixed point of the upper face to the jaw contour. Moreover, wrinkles depth underneath the eyes decreased significantly by 13%.

Globally, we offer a new active ingredient from a *Bacillus* strain from the deep Indian Ocean living under chilling conditions near the exotic Reunion Island which is able to reenergize and strength the skin in a blast to have it ready for daily live challenges.

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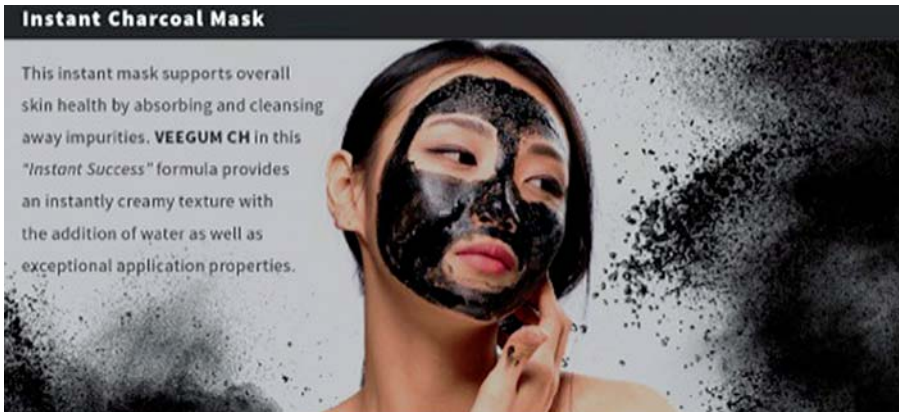


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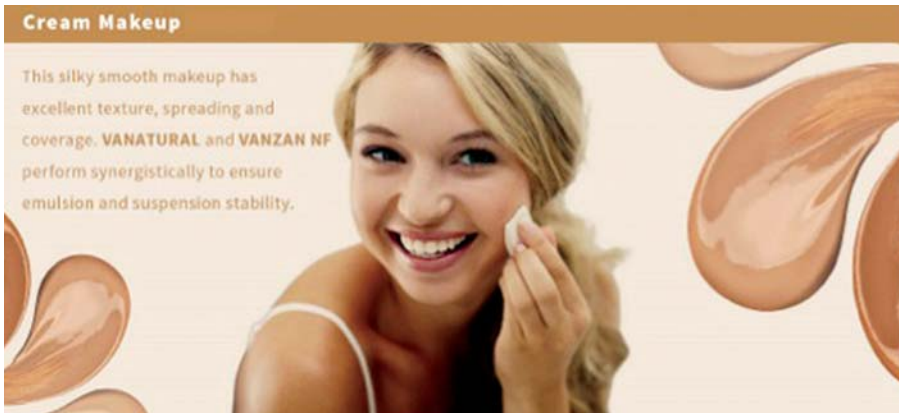


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# Urban life dermopurifying active ingredient from the sea

**by Armelle Bergé[1], Laetitia Cattuzzato[1], Erwan Le Gelebart[2], Jérôme Loeuil[3], Anne-Sophie Dutailly[3]**

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## Introduction

The world's ocean biodiversity is an endless source of new active ingredients. In the past 30 years, the interest has been growing for marine products and especially microalgae for cosmetics, pharmacy, food and bioenergy's applications. Some macro algae of economic interest have been extensively studied and used. Nevertheless, a huge number of macro algae remains little known as they cannot be easily collected in their environment, because of size issue, ephemeral state, epiphytism (living at the surface of another algae) ... To create value from this unexplored biodiversity, Seppic proposes a new technique, developed by Biotechmarine, for macro algae cell culture. The technology starts with isolating macro algae cells from a single drop of marine water, or from colonized shells, rocks or other algae. From just Brittany and more precisely Brehat archipelago remarkable biodiversity, a bank of macro algae cells strains of potential interest has been built. After isolation work to obtain mono-specific cultures, strains are cultivated, at first, in small volumes (in autotrophic conditions), and then through scale-up in order to reach industrial scale (figure 1). One of the main difficulties, in this process, is to keep cells viable during the scale-up. Culture conditions such as salinity, nature of nutrients, mixing, gas introduction, temperature and light are adjusted to obtain an optimal biomass quality.

Then the active molecules, trapped inside cells, are collected thanks to a precise extraction method.

**Figure 1:** Macroalgae cell cultivation, from isolation to freeze dried biomass Thanks to this technology, an unexplored epiphyte red algae Acrochaetium moniliforme (figure 2) has been isolated. Due to its small size (60µm – 2cm) this species has been very little studied and described in the literature. It was identified in collaboration with the French Natural History Museum and the French National Center for Scientific Research (CNRS)

**Figure 2:** Acrochaetium moniliforme (x400) Acrochaetium moniliforme is an epiphytic alga that grows harmlessly

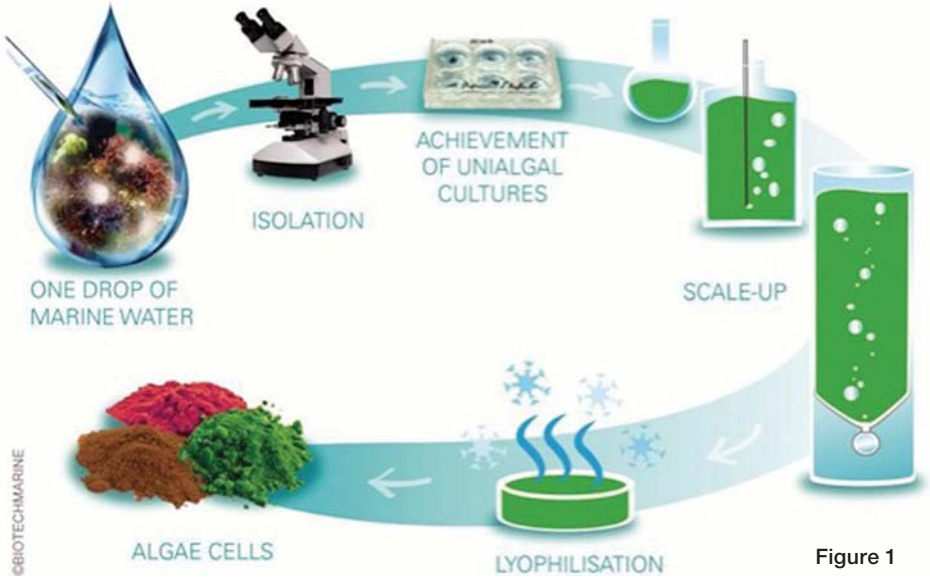


Figure 1

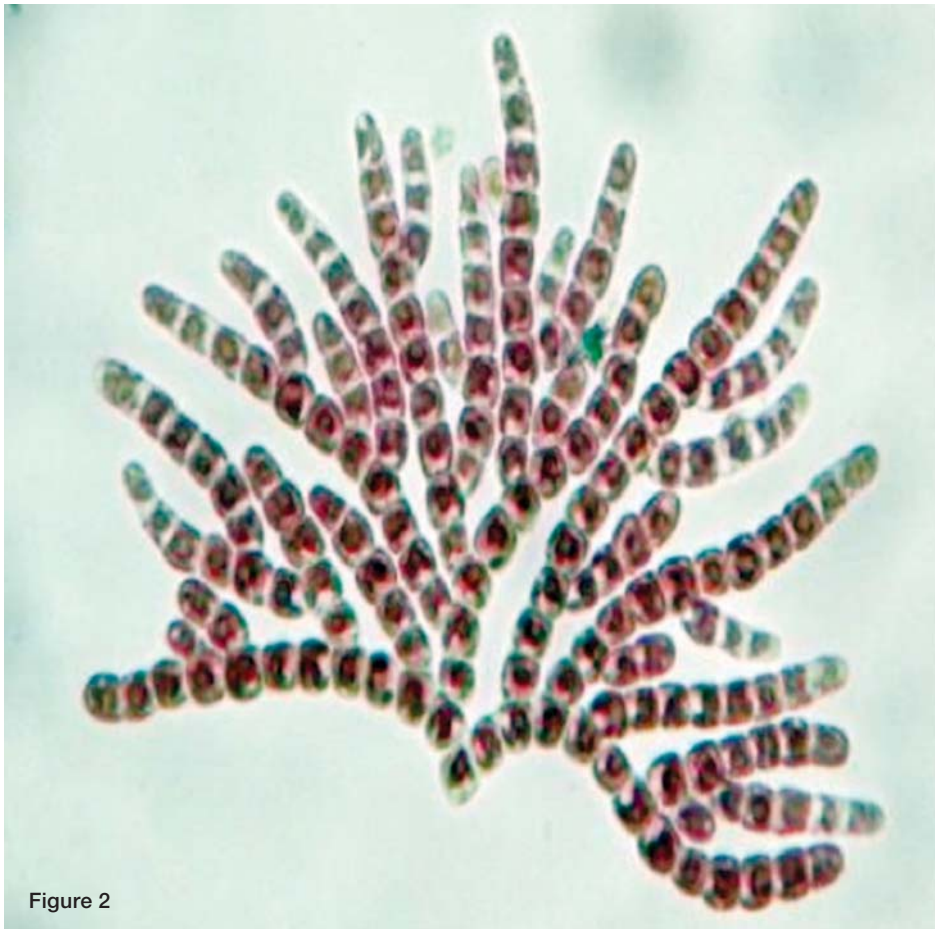

Figure 2

upon other seaweeds using them as a physical support. Acrochaetium species are known to settle as a secondary occupant on the biofilm formed by bacteria and diatoms which are the first colonizers of host algae. In some cases, some close and precise interaction between the host algae and the epiphyte algae has been demonstrated (Weinberger et al., 2007). Micro-organisms and epiphytic algae intervene in the exchanges of matter (nutrients and waste), gas (CO2 and O2) and light energy between the host and the marine environment. They provide their hosts with first-line protection against external aggression. Interested by these properties and exchange capacities, Seppic has decided to study Acrochaetium moniliforme extract and its properties for the skin and has thus discovered the new urban life dermopurifying active ingredient. How to stop the urban pollution impact on the skin Pollution is a global health concern. The effects of systemic exposure to different types of pollutants are now well described, and the effects on skin are of growing concern.

As a physical barrier between the body and the external environment, skin is challenged everyday by atmospheric pollutants. Toxic gases, particulate matter and indoor pollutants (figure 3) are the main pollutants described in our industrial cities and lives (Drakaki et al., 2014).

**Figure 3:** Different classes of atmospheric pollutants and examples (from Valacchi et al., 2012 ; Drakaki et al., 2014 ; Mancebo and Wang, 2015) These pollutants are known to have a negative impact on skin health (Lefebvre et al., 2015 and 2016), leading or contributing to some disorders such as accelerated skin ageing, pigmentation irregularity, acne, atopic dermatitis, skin cancer, psoriasis... (Vierkötter, 2010; Yang et al., 2014 and Roberts et al., 2015). Before these troubles, the first visible impact of daily pollution is oily skin and the increase of sebum production. However, the direct link between pollution and skin lipid production has not been really demonstrated. An innovative in vitro model has been exclusively developed for Seppic to investigate this link between pollution and lipid production applying urban dust on human sebocytes,

It has showed that pollution (urban dust) induces an increase of lipid production by 40% by the sebocytes. This overproduction of lipid was specifically inhibited by Acrochaetium moniliforme extract by 17%. (Figure 4)

**Figure 4:** Regulation of the effects of pollution on sebocytes (in vitro model) This effect is really specific to pollution context as this kind of regulation was not observed without pollution stress. Furthermore, an extract of another the red algae Palmaria palmata, known for its sebum regulator properties, was tested in parallel in this model. It did not show any modulation of lipid production by sebocytes. These data highlight the specificity of the algae Acrochaetium moniliforme against pollution and the interest of the malcro

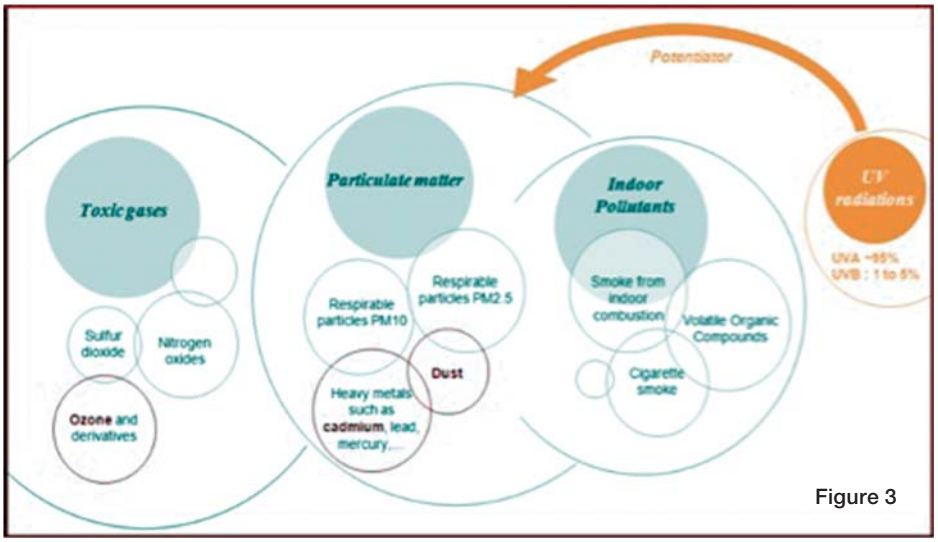


Figure 3

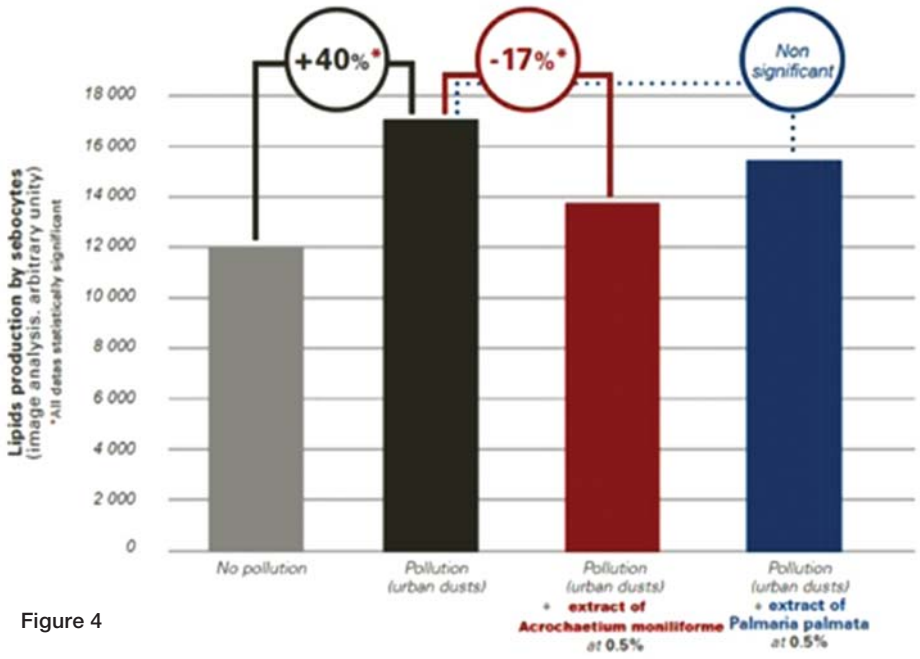


Figure 4

algae cell culture technology. Other mechanisms are induced by pollution such as: - Oxidative stress with generation of free radicals inducing protein carbonylation, DNA damages and lipid peroxidation... - Inflammatory casacades, with an increase in pro-inflammatory mediators such as IL-8, IL- 1 or cyclooxygenase 2, under pollution stress (Ushio et al., 1999; Choi et al., 2011)

To fight these mechanisms, Acrochaetium moniliforme extract is really efficient: on keratinocytes exposed to cadmium it has demonstrated its protective effect on cell viability & metabolism (up to 71%) and inflammatory mediators: regulation of IL-1α (-96%) and IL-8 (-62%). Ozonolyzed Squalene: An innovative pollution marker; a first in Asia

The squalene in sebum is the skin primary means of antioxidant defense (along with vitamin E). Squalene is very sensitive to oxidative stress caused by pollutants such as ozone. It is thus submitted to "ozonolysis", a specific form of oxidation. Thus, ozonolyzed squalene can be used as reliable marker of the impact of pollution on the skin (Wisthaler and Weschler, 2010), by opposition to oxidized squalene which can be generated by many other external stresses, not specifically pollution.

For the first time in Asia, a specific study has been designed to quantify the impact of pollution on the skin by the measurement of ozonolyzed squalene. After 56 days in a polluted environment in Shanghai, Acrochaetium moniliforme extract has been able to reduce ozonolyzed squalene by 61%, confirming the specific protective activity of this algae extract for the skin

Clinical regulation of oily skin in polluted environment

In order to check the visible protection against pollution on volunteers, a clinical study was conducted in Shanghai on 2 groups of 20 Chinese women with oily skin, applying of 1% of the product (or a placebo), twice a day for 56 days. Measurements throughout the period of study indicated a mean of micro particle levels 5 times higher than the recommendations of the World Health Organization (48µg/m3 of PM2.5).

The Acrochaetium moniliforme extract was significantly more efficient than the placebo as it was able to visibly reduces by 34% the sebum of the skin exposed to pollution. (Figure 5)

**Figure 5:** Visible sebum regulation in a polluted atmosphere by Acrochaetium moniliforme extract CONCLUSION Performed tests approached pollution in different mechanisms and showed consistent protective efficacy of Acrochaetium moniliforme extract:

- epidermal cells protection from cadmium
- sebaceous gland protection from urban dust
- skin protection from ozone In vitro data on cadmium-stressed keratinocytes showed an interesting protection capacity of Acrochaetium moniliforme extract on the epidermal mechanical barrier, both protecting directly the cell integrity and downregulating the induction of an inflammatory state. In vitro data on a new specifically designed protocol based on the use of human sebocyte cell line, showed that these cells are highly stimulated by urban dust and react overproducing lipids. These new insights suggest the existence of a specific biological mechanism in oily skin: reactive sebum due to polluted environment. Acrochaetium moniliforme extract was specifically able to reduce this impact.

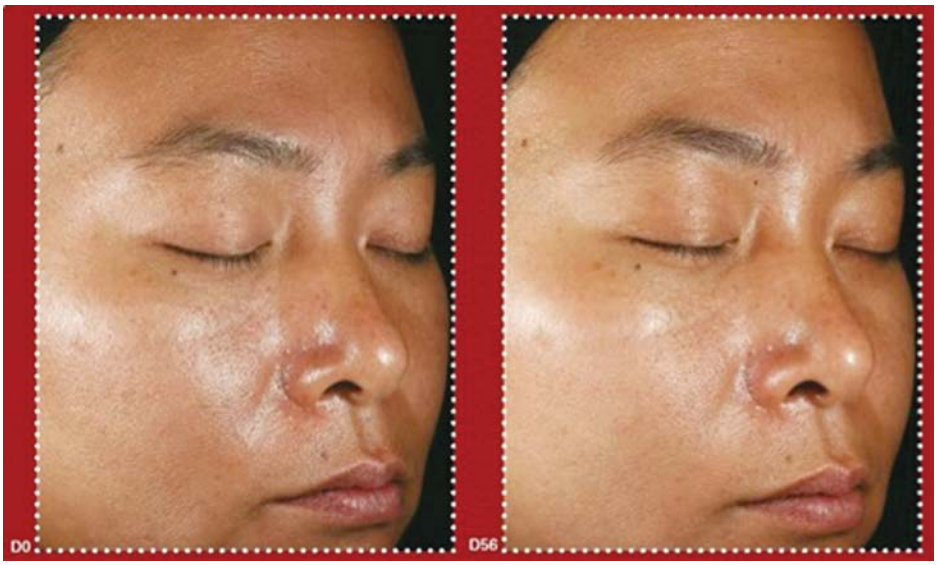


Figure 5

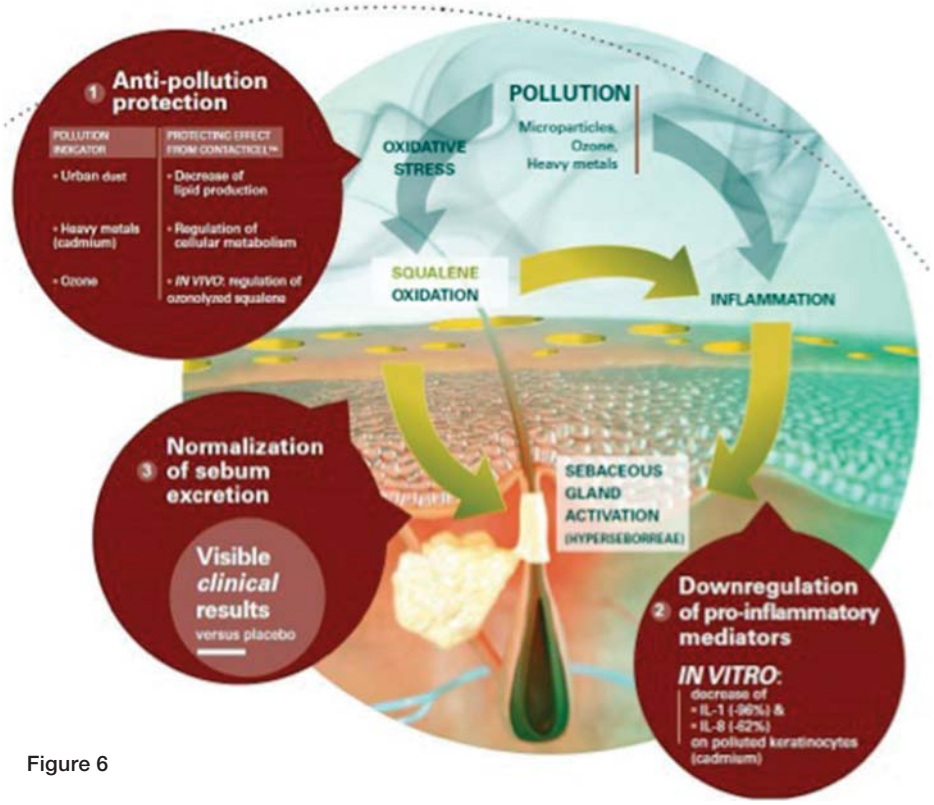


Figure 6

Finally, in vivo data, obtained in a study conducted in an urban polluted environment (Shanghai), confirmed the efficacy of Acrochaetium moniliforme extract, decreasing sebum production (neutral lipids & squalene), modulating the inflammatory state (free fatty acids) and decreasing direct pollution impact on skin (ozonolysed squalene). Thus, through this global protective effect (Figure 6), Acrochaetium moniliforme extract can be proposed as an urban life dermopurifying ingredient in all kind of formulation with anti- pollution claim. Thanks to this unique macro algae cell technology, Seppic is able to create value from this unexplored marine resource and offer it to its customer to get rid of oily skin for long term benefits.

**Figure 6:** Global protective properties of Acrochaetium moniliforme extract on skin

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# Chemical and sensory analysis of an adulterated silicone emulsion

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## Introduction

Silicone emulsions are used in various industrial applications such as food, cosmetics, pharmaceuticals and textiles. These emulsions are heterogeneous systems consisting of an immiscible liquid dispersed in another in the form of droplets, which can have thermodynamic instability [1]. These emulsions are designed to replace organic solvents. Due to its use as a raw material, its quality assurance can mean significant risk reduction in the final product malformations. Small changes in the composition of a raw material can have a negative impact on sensory terms, significantly affecting the quality of a product.

In the particular case of odours, they can have a direct effect on the consumer, since, among other aspects, they can alter the identity of the product. In more specific terms, odours are interpretations of our brain to stimuli caused by volatile substances that reach the sensory receptors of human nose [2]. These substances are relatively small and may belong to different classes of chemical families. In order to be sensorially perceived, odour molecules must overcome a threshold concentration in the air, which varies from one molecule to another. Thus, some molecules can be detected at high concentrations and other from very low concentrations. The latter tend to be associated with unpleasant odours and thus identification can be a difficult task.

Gas chromatography (GC) coupled to a mass spectrometer (MS), referred to as GC-MS, is undoubtedly the most robust and consolidated method in the analysis of volatile organic compounds (VOCs). This technique is able to detect, identify and quantify those VOCs reaching a chemical detector once a sample is introduced into the gas chromatograph. A GC comprises a chromatographic column capable of separating the chemical components of the sample as they progress through it by a carrier gas (usually helium). At the end-of-travel, the diverse (and separated) sample's components reach the chemical detector (MS) at different times, being subsequently detected and identified [3].

The most common GC-MS types are formed by single quadrupole mass spectrometer systems. However, it is possible to obtain more robust, sensitive and higher resolutions analysis. That is the case of mass spectrometry with Time-of-Flight (ToF) detectors, which incorporate faster detection sensitivity and higher resolution with respect to the conventional MS. Despite the fact that these systems are not meant for odour analysis, it is possible to connect a sniffing port to the GC column, so that the compounds can be simultaneously evaluated by the human nose and chemically analysed.

In this study, an odour comparison test by means of GC-Sniffing-ToFMS analytical technique of a silicone emulsion used in personal hygiene products was performed. Two types of samples, a reference emulsion and an adulterated one, were analysed and compared in terms of odours perception and VOCs composition.

## Sampling preparation

A volume of 30 ml of each sample (reference and adulterated silicone emulsions) was used for the analysis (Figure 1a). Samples emulsions were previously stirred and then introduced and confined in a microchamber/ thermal extractor each at the temperature of 35°C (Figure 1b). After 5 minutes under these conditions, an adsorbent tube (Tenax) was inserted on the top of the microchamber to promote the transport of the volatile organic compounds from the headspace to the tube, by means of a flow of nitrogen carrier gas (99.999% purity N2). The acquisition time of the sample in the tube was 5 minutes with a N2 flow of 50 ml/min, being the total collected volume of 250 ml. The sample tube was kept closed with two plugs at its ends until the time of analysis.

Figure 1. a) each type of silicone emulsion (reference and adulterated) samples, b) emulsion samples deposited in the microchambers.

## Experimental procedure

The instrument is composed of a gas chromatograph (Agilent 7890 model, USA), Time-of-Flight mass spectrometer (BenchTOF-dx model, Almsco, Germany) and thermal desorption unity (Unity2, Markes, UK). The amount that is driven off, according to a specific temperature programme, can be selected to optimize the resolution of the analysis.

The desorption tubes with sample were connected to the thermodesorption unit of the GC-ToFMS instrument. They were individually subjected to high temperatures (300°C) during its initial phase to desorb VOC that were captured during sampling. Afterwards, VOCs were entrained by a flow of helium carrier gas (99.9999% purity He) to a cold trap at low temperature (0°C) by thermoelectric cooling, where they were again retained. Then, the cold trap was heated drastically, reaching a maximum temperature of 350°C to release and drag all VOCs into the GC for subsequent chromatographic separation. At the end of the tour of the GC column, once separated, the compounds reached the mass detector at different times, being ionized and by the Time-of-Flight (ToF) selector. Mass of ions were determined with great precision to allow their identification based on the spectral library NIST11.

For the GC-Sniffing analysis, the flow of compounds that finished their route through the column, which in a conventional GC analysis would go to the MS detector, was diverted to a sniffing port (Figure 2). In this way, trained panellists were able to detect and describe the stimuli produced by individually inhaling each compound separated by GC, while they eluted through the sniffing port. Additionally, samples were analysed again for compounds identification and quantification by the mass spectrometer. Thus, the information obtained by both chemical detector (MS) and sensory appraisal (human nose) provided relevant data on the chemicals present in each sample and a description of the associated odour. Odournet panellists are continually evaluated based on internal training routines to improve detection and semantic vocabulary to describe odours.

During GC-Sniffing dynamic, an attribute, intensity (1 to 5) and the time of the analysis was described by the panellist every time an odour was detected (Table 1). Since the runtime analysis took about 45 minutes, the task of 'sniffing' was performed by two panellists' shifts every 15 minutes. Thus, for each analysis (45 min) a panellist smelled 2/3 and the other one 1/3 of the sample. In order that each panellist could smell the full range of compounds, a second analysis was replicated where the order of performance of the panellist was reverse. This process was repeated to give the opportunity to each panellist to smell two times the same sample (Figure 3).

| Value | Meaning | Characteristics |
|---|---|---|
| 1 | Faintly perceived | Sometimes difficult to describe |
| 2 | Perceived | Noticeable |
| 3 | Strong | Very well perceived |
| 4 | Very strong | Nasal saturation |
| 5 | Extremely strong | Necessary to interrupt the analysis (occur only in extreme cases) |

Table 2. Some of the descriptors found in the emulsion samples with their corresponding chemical identification.

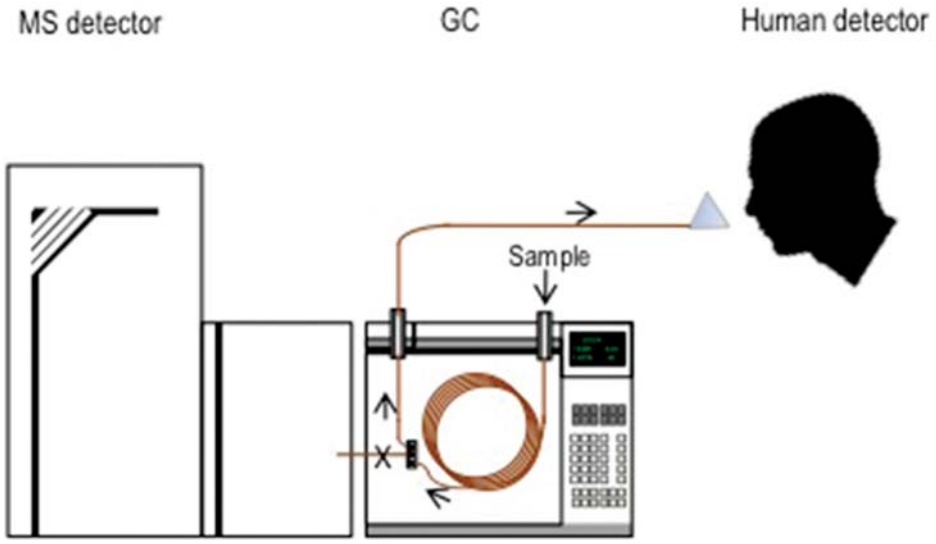


Figure 2. GC-Sniffing analysis configuration. In a standalone run, the flow of VOCs is chromatographically separated during the analysis and each individual compound is then directed towards the nose of an assessor.

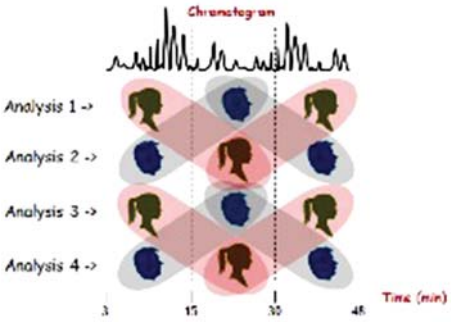


Figure 4. Illustrative image of a panellist performance during a GC-Sniffing analysis.

It is important to note that this type of analysis is relatively complex, requiring a quick reaction of the panellists at the time of evaluation. A number of compounds can bypass the port within

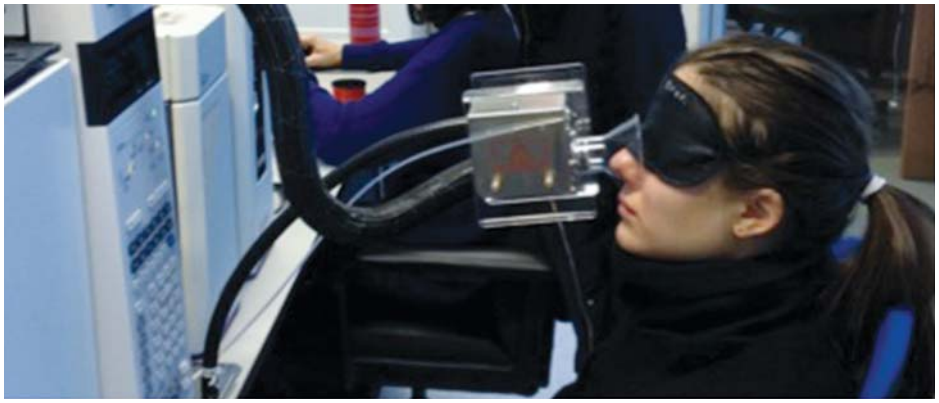

Figure 4. Illustrative image of a panellist performance during a GC-Sniffing analysis.

second intervals. In that sense, as part of the training and analysis of the panel, sample evaluation is carried out blindfolded to avoid any distraction by other stimuli, while a second panellist records the observations of his/her partner (Figure 4).

As for the attributes assigned to odours, Odournet uses its own libraries and reference databases. For each sensory perception, the compound may be responsible for the detected odour is then searched in the chromatogram based on the retention time. This is the time when the compound is detected by both the mass detector as nose analyst. It is frequent in this type of evaluation that some compounds generate a sense of smell (concentration above the threshold of perception) without being detected by the mass detector. This is because the human nose has an even greater sensitivity than the more sensitive detectors.

Another important aspect to consider is that the overall odour of a sample (gas mixture under study), can generate a different perception than that of the compounds when evaluated individually by GC-Sniffing. The compounds may have a synergistic/antagonistic effect (amplify or weaken the odour perception). The link between the general smell of a sample (mixture of compounds) and the odours detected individually is not always straightforward.

The combination of these two techniques of analysis (chemical and sensory) allows to identify and rank the most relevant compounds that generate a perception of smell. In this particular study, sensory perceived compounds were determined by means of an internal library from Odournet using threshold values for olfactory perception based on an extensive literature review and determined by our specialists.

## Results

More than 80 individual odours were detected by sensory evaluation GC-Sniffing in both samples. We selected those odours that had a different sensory assessment or were detected in only one of the samples. Figure 5 represents the odour profile based on sensory differences.

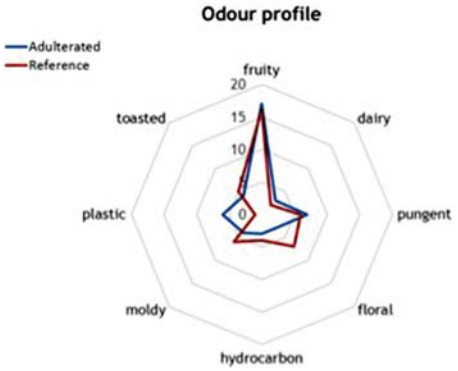


Figure 5. Odour profile representation of both silicone samples, based on sensory odour descriptors and evaluation differences.

Notable differences in plastic and floral notes are depicted in Figure 5, which can be attributed as unpleasant and pleasant respectively. In this sense, it can be seen that the reference sample is favoured by a greater amount of pleasant floral notes and less in unpleasant characters (plastic). This could help identifying which odours are responsible for the sensory differences, between the adulterated and the reference samples, by ToFMS chemical identification and know the chemicals that contribute to these odours. Table 2 shows some of the descriptors found in the analysis of both samples by GC-Sniffing versus time analysis (rt), plus their corresponding chemical identifications.

Of the compounds shown in Table 2, except m,p-xylene and isoamyl acetate, the remainder was identified both sensory and chemically but only in the adulterated sample. This shows that it is possible to reduce the volume of search for potential chemical responsible candidates for the smell of the defective sample.

## Conclusions

By using the analytical technique of GC-Sniffing-ToFMS it has been possible to find differences in sensory and chemical terms in two samples of silicone emulsions, one of them adulterated. The information obtained will narrow the search to only to a subset of odours that might be responsible for the sensory defect of the adulterated sample.

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| rt (min) | Attribute | Compound | CAS No. |
|---|---|---|---|
| 25,8 | medicinal, plastic | m,p-xylene | 108-38-3/106-42-3 |
| 25,9 | banana | Isoamyl acetate | 123-92-2 |
| 30,7 | rubber, paper | 6-methyl 5-Hepten-2-one | 110-93-0 |
| 31,5 | sweet, fruity, green | Limonene | 138-86-3 |
| 31,6 | solvent, fresh | p-Cimene | 99-87-6 |
| 34,1 | fresh, vegetal | p-Cimenene | 1195-32-0 |
| 39,6 | fresh, floral, resine | Geraniol | 106-24-1 |
| 39,8 | rubber, gasoline | Benzothiazol | 95-16-9 |

Table 2. Some of the descriptors found in the emulsion samples with their corresponding chemical identification.

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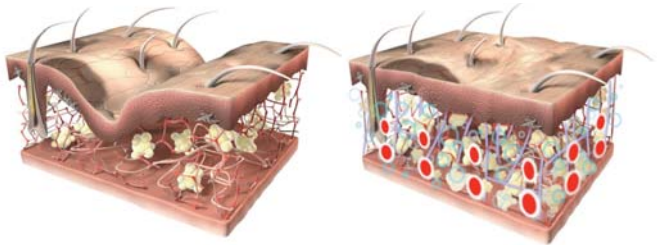

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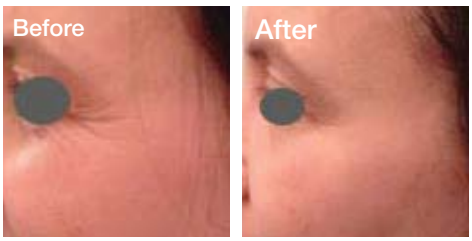



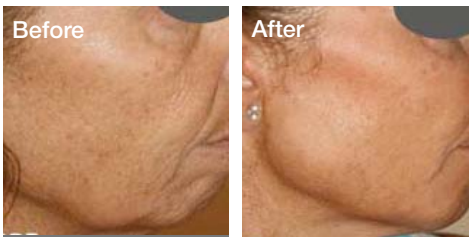


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