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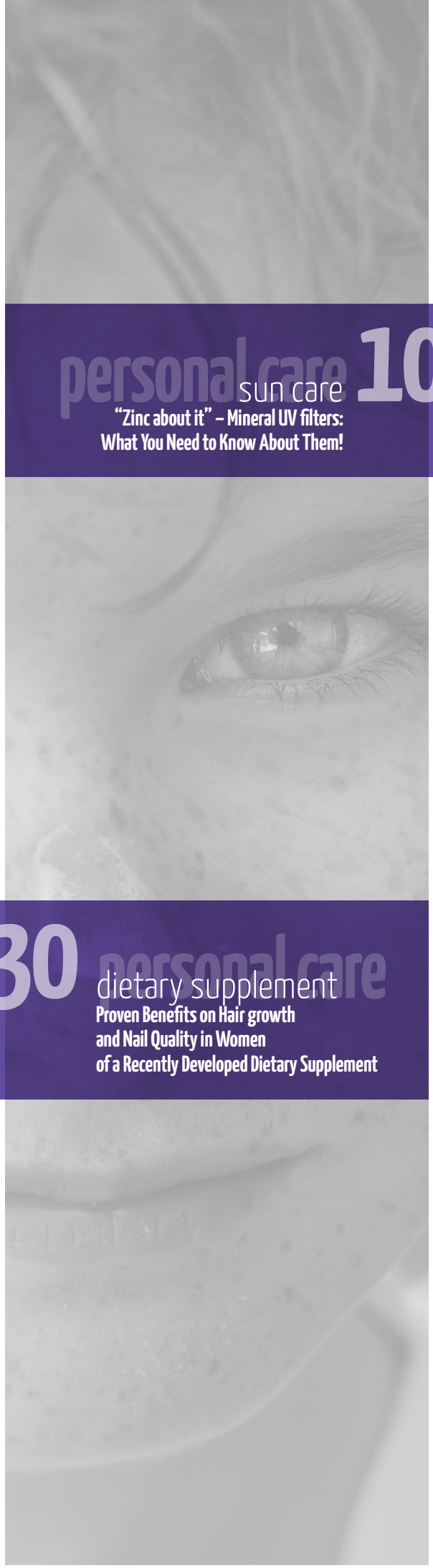

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Nano or Non-Nano: the Key Aspect of the Measurement Method

M. Sohn, W. Wohlleben, P. Müller, D. Botin, J. Giesinger, M. Schnyder, S. Kruś, S. Acker, B. Herzog

abstract

Despite the UV performance benefits of particulate UV filters, concerns have been raised over their potential percutaneous permeation leading to the requirement of a specific approval for all nano UV filters in Europe. Under the legally binding definition of the Cosmetic regulation No. 1223/2009, nanomaterial means […] a material […] on the scale from 1 to 100 nm. The recommendation of the European Commission (2011/696/EU) revised 10th of June 2022 (2022/C229/01) further specifies that a material is nano when at least 50% of the number size distribution of the constituent particles is comprised between 1 and 100 nm. The situation for Titanium Dioxide and Zinc Oxide is confusing since the constituent particles are often part of agglomerates. Some suppliers clearly specify the nano feature of their material, while others declare the non-nano property of their inorganic products. The objective of this study was, therefore, to measure the number-based particle size distribution of three marketed Titanium Dioxide and Zinc Oxide grades using the decision support flow scheme devised by the NanoDefine project. This group was created to support the identification of nanomaterials as required by the European legislation. We employed the tier 1 method asymmetrical flow field-flow fractionation (AF4) coupled with UV and IC-PMS (inductively coupled plasma mass spectroscopy) detectors and tier 2 method transmission electron microscopy (TEM). The study clearly taught that the samples that appeared to be non-nanomaterials with AF4-IC-PMS measurements were finally proven to be nanomaterials using the TEM tier 2 method. Relying only on tier 1 methodologies such as AF4, X-Ray Disc Centrifuge or Dynamic Light Scattering to determine the non-nano property of a material is insufficient, since tier 1 methods are not able to detect constituent particles as part of agglomerates, which is often the case for Titanium Dioxide and Zinc Oxide. The status of being a non-nanomaterial can, in this case, only be certified with a tier 2 technique, particularly electron microscopy.

Introduction

UV filter ingredients used in sun protection products must be approved by the appropriate authority to be used in the respective marketplace; this is true all around the world even if the legislation might differ between the countries. All UV filters absorb UV radiation; filters in particulate form are additionally able to reflect or scatter light. Therefore, it is possible to distinguish between soluble and insoluble or particulate UV filter ingredients. To efficiently absorb UV light, the size of the particles ideally lies in the nano size range [1]. Today, two inorganic materials Titanium Dioxide (TiO_2) and Zinc Oxide (ZnO) and two organic materials Tris-Biphenyl Triazine (TBPT) and Methylene Bis-Benzotriazolyl Tetramethylbutylphenol (MBBT) are approved as nano-particulate UV filters for personal care. A third organic one, Bis-(Diethylaminohydroxybenzoyl Benzoyl) Piperazine, obtained a positive scientific opinion and should be placed on the positive list very soon [2-4]. Particulate filters are commonly used in sunscreens; indeed 42% of sunscreens launched in Europe in 2020 contained at least one of the four registered nano UV filters [5]. Despite their huge benefits in term of performance, their use in sunscreens raised concerns over their potential percutaneous permeation. In Europe, this led to the requirement of a specific approval for all nano particulate UV filters, even for the ones already listed in Annex VI of the Regulation (EC) No. 1223/2009 on cosmetic products, implying a new data submission and a new examination of their safety profile by the Scientific Committee of Consumer Safety (SCCS), which is a commission of independent experts [6]. Under the regulatory definition of the Cosmetic regulation No. 1223/2009 (article 2 (1) (k)), nanomaterial means an insoluble or biopersistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm [7]. To define a nanomaterial more precisely, the European Commission published a recommendation (2011/696/EU) revised 10th of June 2022 (2022/C229/01). In this recommended definition, which has in the meantime been adopted under REACH [8], a nanomaterial means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions are in the size range 1 nm – 100 nm [9]. There are numerous techniques available to measure the size of particles; their range of applicability, however, depends on their measurement principle and the physico-chemical parameters of the tested material. Not every method is appropriate

to measure the size of every particulate ingredient, yet, the analysis result and drawn conclusion may be incorrect due to the use of an irrelevant measurement method. Therefore, the choice of the method highly depends on the characteristics of the nanomaterial to be tested. From the four approved nano particulate UV filters listed in Annex VI of the Regulation (EC) No. 1223/2009, the two organics TBPT and MBBT exist only as nano and are labelled accordingly. They were shown to be fully safe regarding skin dermal permeation and are allowed up to a use concentration of 10% in ready-to-use preparation excluding spray applications or applications posing a risk of inhalation. Regarding the inorganic UV filters, some suppliers clearly specify the nano feature of their material, while others declare the non-nano property of their inorganics. The situation for TiO_2 and ZnO is confusing regarding their particle size distribution since the constituent particles are often part of agglomerates or aggregates. This poses a difficulty since the Regulation (EC) No. 1223/2009 states the need to consider the internal structure, but not every measurement method is able to identify the constituent particles in a material. To support the implementation of the Cosmetic Regulation regarding nanomaterials, the project "NanoDefine" was funded by the EU's 7th Framework Programme for Research [10]. The NanoDefine consortium has developed a decision tree scheme published by the Joint Research Center (JRC) [11] to guide any operator, firstly in the characterization of the particulate material, then in the choice of the most appropriate measurement method to evaluate any material (powder or dispersion) or finished cosmetic product in order to finally identify if the material is nano or non-nano according to the EC definition of nanomaterials [12].

The objective of the present study was to measure the number-based particle size distribution of several marketed Titanium Dioxide and Zinc Oxide grades using the decision support flow scheme devised by the NanoDefine project. To measure the median value of the particle size and the number-based distribution of the test samples, the two methods were employed: asymmetrical flow field-flow fractionation (AF4) coupled with UV and plasma mass spectrometry (ICP-MS) detectors and transmission electron microscopy (TEM).

Materials and methods

Samples of inorganic UV filters

We measured the median value of the particle size and number weighted distribution of different market products of Titanium Dioxide and Zinc Oxide as listed in **Table 1**.

Sample No.	INCI	Product form	Size indication provided by the supplier
1	Titanium Dioxide (and) C12-15 Alkyl Benzoate (and) Polyhydroxystearic Acid (and) Stearic Acid (and) Alumina	Dispersion	179 nm with X Ray Disc Centrifuge
2	Titanium Dioxide (nano) (and) Silica	Powder	(nano) indication given by the supplier
3	Zinc Oxide (and) Titanium Dioxide (and) Silica	Powder	Average particle size 455 nm (DLS)*

* Dynamic Light Scattering

Table 1: Investigated Titanium Dioxide and Zinc Oxide market grades.

Sunscreen market product

In parallel, we also evaluated a market product in the form of an emulsion containing Zinc Oxide as solely UV filter. The sunscreen product claimed a SPF of 50+; in the INCI declaration the Zinc Oxide was not listed as a nano UV filter.

Particle size measurement methods

The NanoDefine consortium was founded to support the implementation of the EC definition on nanomaterials. It included European RTD performers, experts from metrology institutes, nanomaterial suppliers, instrument manufacturers, regulators as well as academics. The objective of the group was to develop an approach which allows to identify whether a material is nano or not according to the EC definition using only robust, readily available and standardized methods to provide a reliable analysis of the number-based size distribution. The resulting list exhibits tier 1 (screening purposes) and tier 2 (confirmatory purposes) methods [13].

In our study, we used two complementary measurement methodologies for each material: asymmetrical flow field-flow fractionation coupled to inductively coupled plasma mass spectrometry (ICP-MS) or UV detector as a tier 1 method and Transmission Electron Microscopy (TEM) as a tier 2 method. The two methods are appropriate to evaluate inorganic materials in the relevant nano size range and provide number-sized distribution of the measured materials. Besides the suitability of the methodology itself, the sample preparation is a core aspect to achieve an accurate result and should not modify the size distribution of the original material. Therefore, recommendations on sample preparation exist using standard operation procedures [14].

Asymmetrical flow field-flow fractionation (AF4)

The asymmetrical flow field-flow fractionation (AF4) system (Eclipse separation system AF4, Wyatt Technology, USA) with a trapezoidal channel with a nominal thickness of 350 µM (spacer W350 µm, Wyatt Technology, USA) fitted with a regenerated cellulose membrane with 10 kDa cutoff (PLGC, Reg. Cellulose 10 kD, Millipore, USA) was used. The AF4 was coupled to an UV spectrometer (DAD 1290 Infinity II, Agilent Technologies) and an ICP-MS (NexION 2000, Perkin Elmer, USA) detector. In this technique, the fractionation occurs in a trapezoidal channel with a semipermeable membrane at the bottom of the channel, called the accumulation wall. After sample injection, the solvent flow transports the sample particle species to the outlet and detectors. The solvent flow has a parabolic velocity profile with the highest velocity at the mid-height of the channel. The fractionation is performed by applying a cross flow perpendicular to the channel flow, which pushes the sample particle species towards the accumulation wall and leaves the channel through the semipermeable membrane. At the same time, the Brownian motion of particles opposes the drag of the cross flow, resulting in the distribution of the sample particles along the vertical axis of the channel. The shape of distribution is then defined by the interplay between the cross flow and the particle diffusion coefficient, D, the latter being inversely proportional to the particle hydrodynamic radius. Therefore, the particle species with smaller radii (larger diffusion coefficients) are further away from the accumulation wall, transported faster and elute first, while the bigger particle species (smaller diffusion coefficients) elute later. The separated particles are then detected using an appropriate detector. The AF4/UV data were analyzed with Astra software, version 7.3 (Wyatt Technology, USA). The ICP-MS data acquisition and analysis were performed using Syngistix software, version 2.4 (Perkin Elmer, USA). The methodology provides a mass-weighted distribution of the particle size, which can be converted to a number-weighted size distribution. The method also provides information on the chemical composition of the particle material via the ICP-MS detector. The AF4 with both detectors was thoroughly evaluated using the NanoDefine Method Manual [11,16]. The calibration of the elution times as function of particle size was done according to the polystyrene size calibration standards (22 ± 2 nm, 51 ± 3 nm, 100 ± 4 nm, 203 ± 5nm and 345 ± 7 nm, Nanosphere Size Standards, Thermo Scientific, USA).

The powder samples (sample 2 and sample 3, **Table 1**) were prepared in sodium hexametaphosphate solution (c = 2 g/l) [15] to obtain a sample concentration of c = 0.5 g/l according to the standardized dispersion protocols for high priority material groups (Technical Report D2.3 (6.9)). The Titanium Dioxide dispersion (sample 1) and the market sunscreen emulsion were dispersed in a water solution of Triton X-100 (MP Biomedicals, Irvine, CA, USA) (c = 5 g/l) to obtain a sample concentration of c = 10 g/l. The dispersions were then sonicated (Branson 550 Sonifier, Model 102C converter, Branson Ultrasonics) twice for 15 minutes at 60% of amplitude with a 5 minute pause, and finally purified passing through a 5,0 µm filter before injection into the AF4 setup.

Transmission Electron Microscopy (TEM)

Samples for TEM were prepared on ultra-thin carbon-coated TEM grid carriers (ECF200-Cu-50 from Science Services GmbH Munich, Germany). The Titanium Dioxide dispersion (sample 1, table I) was dispersed 1:100 in a water solution of 50 mg/L Triton X-100. The two powder samples (sample 2 and sample 3, **Table 1**) and market sunscreen emulsion were dispersed 1:100 in ethanol. In addition, for the sunscreen emulsion, the inorganic content was separated from the organic oil content by centrifugation using an Eppendorf Mini-Spin at 13400 rpm (Eppendorf DE, Hamburg, Germany). The Titanium Dioxide dispersion was analyzed with a Themis Z3.1 TEM (Thermo-Fisher, Waltham, USA); the three other samples with a Tecnai Osiris F200 TEM (Thermo-Fisher, Waltham, USA) in bright-field as well as HAADF-STEM (High-Angle Annular Dark Field with Scanning Transmission Electron Microscope) mode. Both microscopes are equipped with a Super-X detector for Energy-Dispersive X-ray Spectroscopy (EDS) for chemical analysis. The data were analyzed using the Thermo-Fisher Velox 2.1x and Bruker Esprit (Bruker, Billerica, USA, version 2) software packages.

Results

The nano range conventionally referred to a size ranging from 1 to 100nm, corresponding to the scale at which nano-related incidences are most expected to take place. The relevance of taking into account the size of the constituent particles in the definition of the Cosmetic Regulation EC 1223/2009 was explained by the fact that, over time, primary particles may be released from the agglomerates or aggregates due to condition changes as stated in the EC recommendation (2011/696/EU) [6]. The EC recommendation (2011/696/EU) revised June 10th 2022 furthermore states that a material is a nanomaterial when 50% or more of the particles of the material exhibit one or more external dimensions in the size range 1 nm – 100 nm in the number size distribution. The quantitative criterium enables the use of the definition in a regulatory context and became a common basis for regulatory purposes; indeed, the classification of a material as nano has consequences on its approval requirements. Therefore, the identification as to whether a material is nano or not is of prime importance.

Asymmetrical flow field-flow fractionation

The elution fractions were analyzed with two different detectors, the ICP-MS and UV detectors. The ICP-MS detector is element specific and allows to detect the presence of the metal ion in each eluting fraction, i.e. in our study Titanium or Zinc. The UV detector allows to detect UV absorbing species in investigated samples and, furthermore, serves for the establishment of the correlation between elution time and particle size of the calibration latex standards.

Figure 1 displays the result of different sizes of reference polystyrene latex particles (20 nm, 50 nm, 100 nm, 200 nm) to establish the correlation between the elution time from the channel and the corresponding size of the particles.

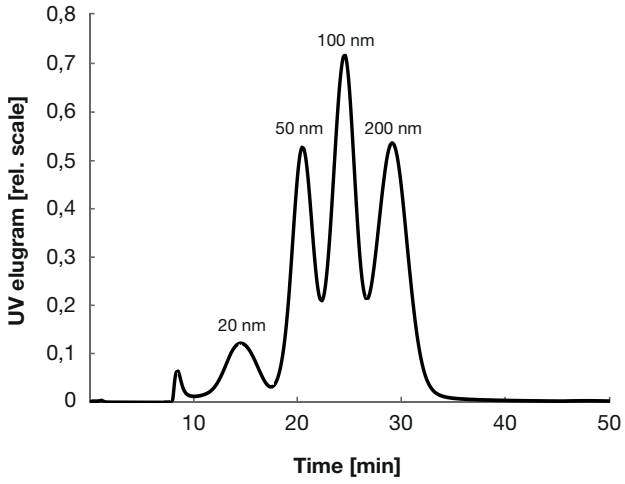

Fig. 1 Calibration of elution time and particle size using polystyrene calibration standards.

In the illustrative example presented in **Figure 1**, a latex particle with a size of 50 nm is eluted and detected after 20 minutes.

The results of the AF4 measurements coupled with UV or ICP-MS detectors are obtained in the form of elugrams; **Figure 2** displays the elugram of the Titanium Dioxide dispersion (sample 1) as an example.

The X-axis corresponds to the detection time of the particles after they were separated by asymmetric flow field-flow fractionation and left the channel. The smaller the particles, the faster they leave the channel and are detected. The scatter blue curve (upper curve) corresponds to the signal from Titanium as recorded by the ICP-MS detector, and corresponds in our sample to the Titanium Dioxide particles. Titanium is detected after an elution time of 12 – 16 minutes and again after 20 – 35 minutes. The solid black line corresponds to the signal recorded by the UV detector, and overlaps the ICP-MS signal with a detection of UV absorbing species after an elution time of 13 – 16 minutes and a second population between 20 – 30 minutes corresponding to the Titanium Dioxide particles. According to the calibration with latex standards **(Figure 1)**, an elution time of 12 – 16 minutes corresponds to particles with a size of less than 22 nm; the elution time between 20 and 35 minutes of the second population species of Titanium Dioxide corresponds to particles with a size ranging from 50 to 200 nm. Assuming the measurement is representative for the complete sample and the particles are spherical and knowing the density of Titanium Dioxide and Zinc Oxide,

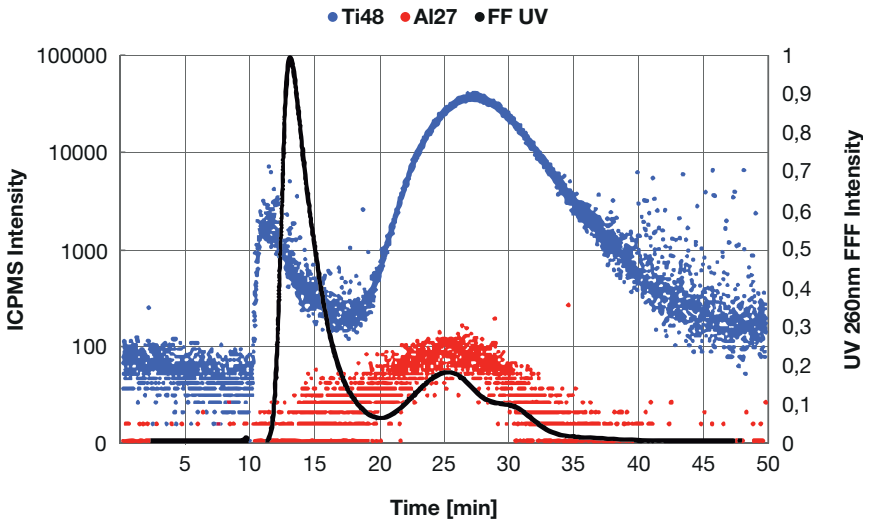

Fig. 2 Elugram of Titanium Dioxide dispersion (Titanium Dioxide (and) C12-15 Alkyl Benzoate (and) Polyhydroxystearic Acid (and) Stearic Acid (and) Alumina). The solid black line corresponds to the signal detected by the UV detector; the scatter blue (upper) and red (bottom) curves correspond to the signals detected by the ICP-MS detector for the element Titanium and Aluminum, respectively.

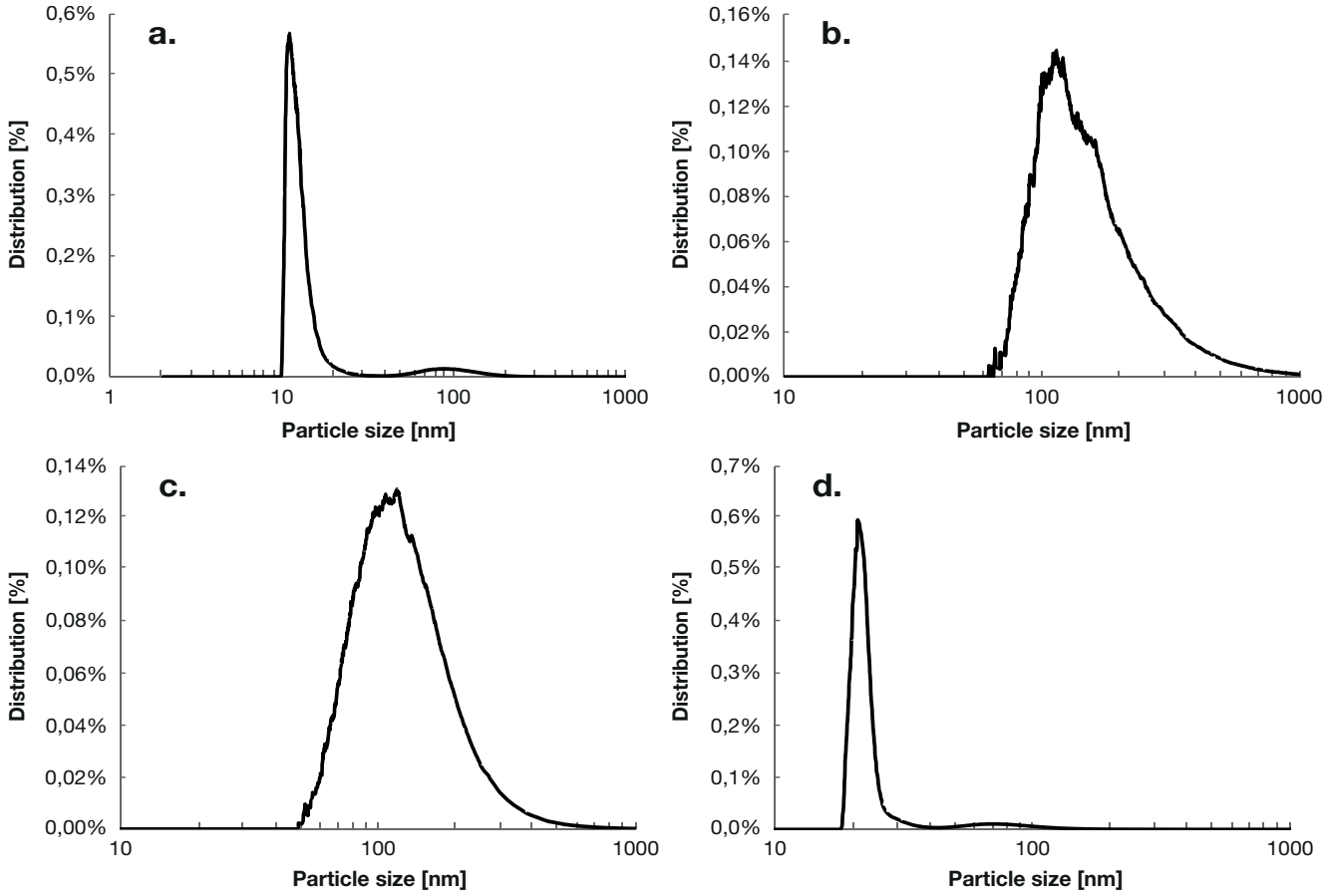

Fig. 3 Number--based particle size distribution using AF4 ICP-MS of: **a**. sample 1 (Titanium Dioxide dispersion); **b.** sample 2 (Titanium Dioxide powder); **c.** Titanium Dioxide in sample 3; **d.** Zinc Oxide in the sunscreen market emulsion.

the mass-weighted particle size distribution can be converted into a number-weighted particle size distribution. The results for each sample are displayed in **Figure 3**.

Table 2 summarizes the number-based median particle size obtained by AF4 measurements and the size indication provided by the supplier.

The AF4-based result classifies Titanium Dioxide dispersion (sample 1) as nanomaterial with a median particle size of 13 nm, which is in discrepancy with the data provided by the supplier claiming a particle size of 179 nm and no indication of which parameter the value of 179 nm is exactly referring to (median, mean…). Also, the Zinc Oxide contained in the market product is measured as being nano, however, the INCI list for that product does not mention the nano feature of the Zinc Oxide. The Titanium Dioxide particles of sample 2 and 3 show a median size greater than 100 nm and both would be classified as non-nanomaterials. The supplier of sample 2, however, mentions the nano status of this Titanium Dioxide grade. The reason of the discrepancy between the AF4 measurements and the nano status claimed by the supplier could be due to the presence of agglomerates or aggregates in sample 2. These discrepancies reveal the complexity of measuring an accurate particle size. In fact, from all methods referenced in part 2 of the NanoDefine Methods Manual [16] and providing a number-based distribution of the size, only electron microscopy techniques can identify and count the constituent particles in aggregates/agglomerates. All other counting methods cannot distinguish be-

Sample	Tested inorganic material	Median (D50) (nm) measured by AF4	Size indication provided by the supplier
Dispersion (sample 1)	Titanium Dioxide	13 nm	179 nm with X Ray Disc Centrifuge
Powder (sample 2)	Titanium Dioxide	137 nm	(nano) indication given by supplier
Powder (sample 3)	Titanium Dioxide	120 nm	Average particle size 455 nm (DLS)*
Sunscreen market emulsion	Zinc Oxide	22 nm	Zinc Oxide was not listed as a nano UV filter in the INCI

* Dynamic Light Scattering

Table 2: Median particle size values (AF4 measurements) and size indication provided by the supplier.

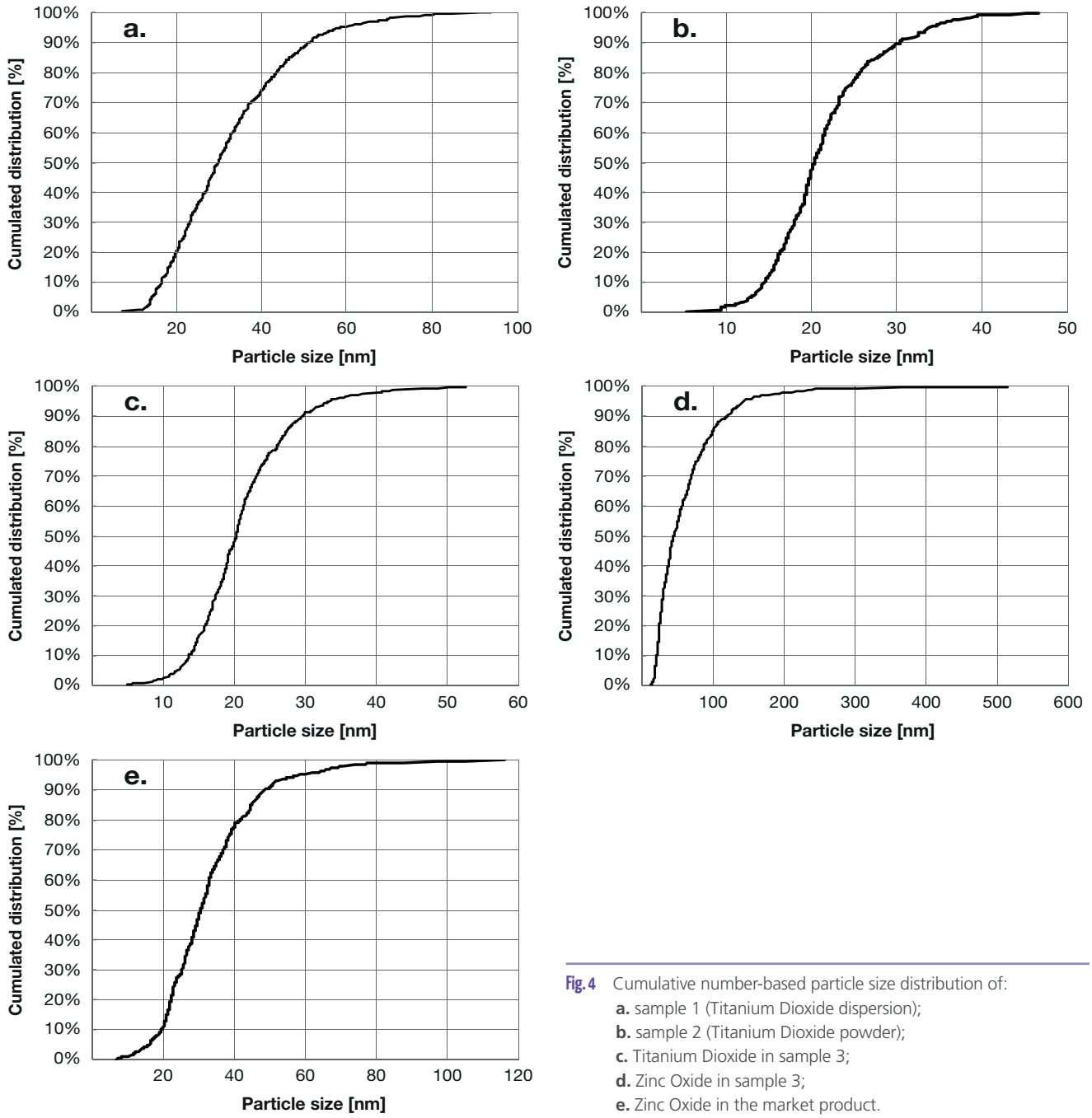

Fig. 4 Cumulative number-based particle size distribution of:
a. sample 1 (Titanium Dioxide dispersion);
b. sample 2 (Titanium Dioxide powder);
c. Titanium Dioxide in sample 3;
d. Zinc Oxide in sample 3;
e. Zinc Oxide in the market product.

tween large particles and agglomerates, consisting of smaller primary particles, both implying a risk of misinterpretation and of classifying a nano material as non-nano material. If the employed method provides a mean or median size of particles below 100 nm, it can be concluded that the tested material is a nanomaterial. This is the case for Titanium Dioxide dispersion (sample 1). The fact that the supplier of sample 1 provides a particle size of 179 nm is most probably due to the X-Ray Disc Centrifuge method used, which is not able to detect the constituent Titanium Dioxide particles in agglomerates/aggregates. The reason for this could be the sample preparation, which was not thorough enough to individualize the constituent particles. Concerning the market product, the producer most probably relied on the information provided by the supplier of the Zinc Oxide, which assumably also wrongly characterized its ingredient. As there remains uncertainty when using a tier 1 method, a result which indicates that a material is non-nano should then be confirmed using a tier 2 technique such as Electron Microscopy. Therefore, all samples in our study were further evaluated with TEM.

Transmission Electron Microscopy

TEM is a counting methodology and belongs to the tier 2 confirmatory methods. It is adapted to the detection and counting of the particles with sizes ranging from 1 nm to more than 1 µm. For each evaluated particle, the smallest dimension (ferret my) was considered. **Figure 4** displays the cumulative number-based size distribution of the TEM evaluation for all samples.

Table 3 summarizes the number metrics (number of particles analyzed, median value of the size distribution, percentage of particles with sizes smaller than 100 nm).

Figure 5 shows a representative TEM image of the dispersion Titanium Dioxide (and) C12-15 Alkyl Benzoate (and) Polyhydroxystearic Acid (and) Stearic Acid (and) Alumina as an illustrative example.

Sample	Form		Number of particles analyzed	Median (D50) (nm)	Particles < 100 nm (%)
1	Dispersion		870	28.0	100.0%
2	Powder		425	20.4	100.0%
3	Powder	TiO_2	477	19.9	100.0%
		ZnO	564	42.8	86.2%
4	Sunscreen market emulsion		509	30.0	99.6%

Table 3: TEM number metrics of tested samples.

The TEM evaluation revealed that the Titanium Dioxide and Zinc Oxide particles of all investigated samples are nanomaterials; the median size value of each being well below 100 nm; for almost all samples, even 100% of the particles lie below the threshold of 100 nm. The difference in the particle size values between tier 1 and tier 2 methodologies for sample 2 and 3 suggests the presence of agglomeration or aggregates and underlines the importance of the correct selection of methodology and sample preparation. Methods which are based on the motion of the particles in a medium tend to measure the size of the aggregates rather than that of the primary particles, since the aggregates are the moving entities [17]. Such methods include Dynamic Light Scattering where the diffusion of the particles is analyzed; also centrifugation and field-flow fractionation where particles move in a centrifugal force field and flow field, respectively.

This study reveals that limiting the nano interpretation of a material to a tier 1 methodology such as AF4, X-Ray Disc Centrifuge or Dynamic Light Scattering is clearly insufficient in the case of Titanium Dioxide or Zinc Oxide UV filters, and leads to an erroneous interpretation and classification of the ingredient as a non-nano material.

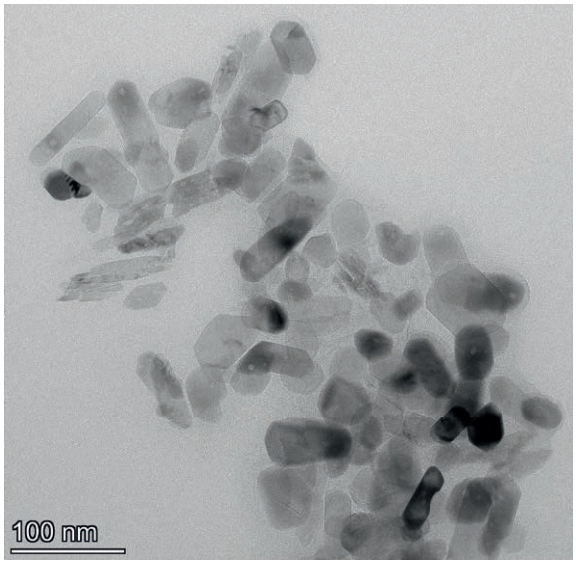

Fig. 5 TEM image of the Titanium Dioxide dispersion (sample 1).

Conclusion

In the present study we applied the decision support flow scheme devised by the NanoDefine project to identify if investigated materials are nano or non-nano according to the EC definition of nanomaterials. We determined the median value of the particle size and number-based size distribution of several marketed Titanium Dioxide and Zinc Oxide products. The study revealed that when using only a tier 1 methodology, a sample may erroneously appear to be a non-nanomaterial, which may result in misinterpretation. This is explained by the fact that tier 1 methods are not able to detect constituent particles as part of agglomerates. Two samples of Titanium Dioxide tested in the present study appeared to be non-nano with AF4 measurements, but were finally proven to be nanomaterials using the TEM tier 2 method. For these reasons, the non-nano statement provided by a supplier who relies only on a tier 1 method, such as X-Ray Disc Centrifuge or Dynamic Light Scattering, should be doubted until it is verified by using an electron microscopy tier 2 technique.

Acknowlegments:

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authors

Myriam Sohn [a], Wendel Wohlleben [b], Philipp Müller [b], Denis Botin [b], Marcel Schnyder [a], Stanisław Kruś [a], Stephanie Acker [a], Bernd Herzog [a]

[a] BASF Grenzach GmbH | Köchlinstrasse 1 | 79639 Grenzach-Wyhlen, Germany

[b] BASF SE | Carl-Bosch-Strasse 38 | 67056 Ludwigshafen, Germany

Corresponding author: myriam.sohn@basf.com

"Zinc about it" – Mineral UV filters: What You Need to Know About Them!

Y. Shao, D. Schlossman, M. Busch, E. Suess

Sun Care Market move towards mineral ingredients

The market for cosmetic products containing sunscreens is growing as consumers become more aware of the harmful effects of UV rays on the skin. The skin is our first layer of protection against the environment. It is essential for our health and forms the barrier that protects us from physical and biological noxae. The greatest physical threat is UV radiation from the sun, recognized as a significant factor in the development of skin cancer [1]. UVB rays are absorbed by the epidermis of the skin and are mainly associated with sunburn. UVA rays that reach the skin penetrate the epidermis and are responsible for skin aging. More and more consumers start considering skin aging and skin cancer as potential damage caused by UV light and become aware of the need to apply sun protection as part of their daily habit as well as summer and holiday routine. At the same time they also start thinking about potential safety issues of UV filters.

There has been an increased demand for safe, environmentally friendly, and effective UV filters that have no negative impact on the environment and human health, leading to pressure on conventional organic UV filters. More and more data are being requested by various authorities to prove their safety for human health, as well as for the environment. The launch of sunscreen products containing the mineral UV filters Titanium Dioxide (TiO_2) and Zinc Oxide (ZnO) is increasing, and more and more cosmetic brands are following this trend to support consumer interest in mineral sunscreens. Claims such as "reef safe," "ocean friendly," and "planet friendly" are appearing on sunscreens. This trend is exacerbated by some local regulations: in some countries, laws are coming into effect to ban the sale, offering and distribution of sunscreens containing the chemicals Oxybenzone and Octinoxate to help preserve marine ecosystems and protect coral reefs: Palau (Western Pacific), the U.S. Virgin Islands, Bonaire and Aruba, Hawaii, and Maui County also passed legislation (Bill 135) banning non-mineral sunscreens and allowing only mineral sunscreens beginning in October 2022. In addition, there are restrictions on innovation due to the strict regulations:

In the USA, no new UV filter has been approved for 20 years, and in the EU, although there is a wider range of UV filters, regulators are also blocking the introduction of new UV active ingredients.

The global mineral sunscreens market is registering a CAGR of over 5% between 2020 and 2030 [3]. In 2018, 45.1% of sunscreen launches contained mineral UV filters compared to 51.6% in 2022 **(Figure1)**. In the US, growth in Google searches for "mineral sunscreen" is +36.4% year-over-year [4]. The currently preferred mineral UV filter is ZnO.

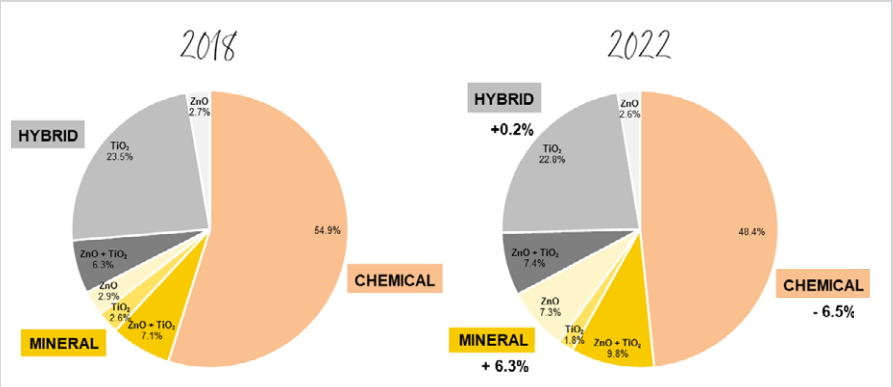

Fig. 1 Market evolution of mineral, chemical and hybrid sunscreens [10]

Mineral UV filters: What you need to know about them!

Basics

Mineral UV filters like TiO_2 and ZnO are particulates. They are comprised of primary particles, aggregates (of primary particles), and agglomerates (of aggregates). Their powder forms available on the marketplace are agglomerated, typically having a particle size between 1- 5 µm. Unlike organic UV filters

that offer UV protection through absorption, mineral UV filters attenuate UV light through both absorption and scattering. TiO_2 attenuates UV light predominately through absorption from 290 to 350 nm, predominately through scattering from above 375 nm, and through both actions between 350 – 375 nm [5,6]. ZnO attenuates UV light through absorption from 290 to 380 nm, and mostly through scattering from 380 to 400 nm [6]. TiO_2 found in sunscreens is usually rutile and has a refractive index of 2.76. ZnO, on the hand, has a lower refractive index of 1.99. Because of the lower refractive index, ZnO in skin care and beach products can be rubbed into the skin and has an acceptable transparency among most consumers, even those with deeper and richer skin tones. This is different from TiO_2, which often leaves behind a whitish or bluish cast. Color cosmetics and BB creams can more readily tolerate the use of TiO_2 as a mineral UV filter because some opacity of the formulation is often desirable and that the iron oxides typically included in the formulation can mitigate its bluish cast.

It is very important to understand that there is a strong correlation between the particle size of a mineral UV filter in a sunscreen and its UV protection efficacy. Therefore, the particle size of a mineral UV filter must be optimized for a sunscreen to achieve broad spectrum protection. TiO_2 attenuates UVB light (290-320 nm) mostly through absorption when its primary particle size is small (10-20 nm). Due to its small size, the scattering of UV light especially UVA light is weak, which results in low UVA protection. Medium particle size TiO_2 (35-70 nm) will absorb effectively all UVB light and light to around 350 nm in UVA. Such TiO_2 will strongly scatter UV light beyond 350 nm, thus providing strong UVA protection. Hence, it is possible to achieve broad spectrum high SPF using only one grade of such TiO_2. However, the downside of TiO_2 in this size range is the white cast. Thus, beach or face formulas created with TiO_2 alone are often tinted with iron oxides to serve customers with diverse skin tones. Dispersion technology is able to reduce, but not eliminate, the white cast caused by the high refractive index of TiO_2.

As previously stated, ZnO absorbs UV light beyond 370 nm to 380 nm. Thus, it is an ideal sunscreen active to formulate with to achieve broad spectrum protection. Like TiO_2, the ZnO's UV attenuation shifts to shorter wavelengths when its primary particle size decreases. Remarkably, the range of primary particle sizes of ZnO from around 20 nm to 250 nm can enable formulations with critical wavelengths ranging from 365 nm to greater than 380 nm.

Deep dive into particle size

A skilled formulator has the ability to manipulate the particle size of mineral UV filters to achieve the desired optical property. TiO_2 and ZnO are typically treated with a hydrophobic coating prior to use. In the EU, the use of coatings is regulated for nano TiO_2 and ZnO. Typical hydrophobic coatings include triethoxy caprylyl silane and dimethicone for ZnO, and dimethicone and aluminum stearate for TiO_2, the latter being derived from natural origin. The hydrophobic coating improves the dispersibility of TiO_2 and ZnO powders in the oils. In general, dispersions of treated TiO_2 and ZnO are recommended for formulation since the dispersions enable the formulator to maintain the particle size in the formula from a lab scale to an industrial scale, batch after batch. Additionally, by varying the surface treatment, carrier, the powder concentration, and the milling process, the particle size of TiO_2 or ZnO can be precisely controlled to be in the range for the desired UV attenuation. To work with powders, the formulator must be able to have production equipment for high shear milling and the ability to control the particle size during the manufacturing process.

The particle size of TiO_2 or ZnO in the dispersion is typically measured using a light scattering size analyzer. Generally for ZnO, a primary size of around 35 nm or dispersion particle size of at least 170 nm is effective for meeting most UVA protection standards. However, when the ZnO concentration in the formulation is 20 to 25%, smaller particle sizes may also be effective for achieving a critical wavelength greater than 370 nm. To achieve a very high UVA/UVB ratio or critical wavelength, ZnO of a larger size, especially non-nano grade with particles between 250-500 nm, is useful. A critical wavelength greater than 380 nm is achievable using non-nano ZnO.

The UVB attenuation of ZnO is less than that of TiO_2, corresponding to a lower SPF per active weight percent. The typical SPF range using ZnO and TiO_2 are 1-2 and 2-3 SPF units per active weight, respectively. However, ZnO provides a higher *in-vivo* PA/SPF ratio that reaches maxima when the primary particle size is in a range of 40 - 60 nm. The PA score of 20 nm ZnO can be kept reasonably high by avoiding excessive size reduction in milling its dispersion.

How to choose the right mineral UV filters for your formulation

The formulator must decide between powder and dispersion. Additionally, the regulatory requirements of the markets where the product is going to be sold must be considered. To go deeper, what the formulator wants to claim, how their regulatory and safety departments look at the subject, and their views on the claims are all important. The next consideration would be whether the formulation should be natural or whether certain ingredients should be avoided. Furthermore, requirements in terms of targeted SPF and UVA protection will dictate the particle size selection and whether zinc oxide should be used alone, in combination with TiO_2, or with other filters. Finally, in case of dispersions, the carrier is important. When dispersions are considered, there are different carriers with different spreading abilities, different sensories, and different volatilities. If the sunscreen actives in the formulation must be used at a high level, which will often be the case for a high SPF formulation, their dispersions should have high active content as well.

Furthermore, inclusive sun care is needed and many customers do not accept sunscreens that leave a white cast on their skin anymore. As mentioned earlier, ZnO is much more transparent than TiO_2. ZnO formulations greater than SPF 30 can appear natural and transparent on skin types four to six on the Fitzpatrick scale. And for those with the deepest and richest skin tones, the inclusion of transparent iron oxides will neutralize the slight milky appearance coming from a high level of zinc oxide. In short, mineral UV filters can be included in sun care formulations that meet the needs of all types of skin tones.

Mineral UV filters: Regulations

Sunscreens are regulated as OTC drugs in the USA. TiO_2 and ZnO are listed by the FDA as Category sunscreen active ingredients that are generally recognized as safe and effective (GRASE). Both the United States and the EU permit TiO2 and ZnO usage up to 25% as sunscreen actives **(Table 1)**.

Region	TiO_2	ZnO
US	≤25 %	≤ 25 %
Canada	≤ 25 %	≤ 25 %
EU	≤ 25 %	≤ 25%
Australia /New Zealand	≤ 25 %	No limit
Japan	No limit	No limit
China	≤ 25 %	≤ 25 %
India	≤ 25 %	not listed
ASEAN	≤ 25 %	≤ 25 %

Table 1: Global Use Limit of Mineral Filters in Sunscreens [11]

Sun care products have to protect against both UVA and UVB radiation to claim broad spectrum protection, although regulations vary among regions. The FDA requires SPF of 15 and a critical wavelength of at least 370 nm to claim broad spectrum protection. Critical wavelength means the wavelength equal to the area under the absorption curve representing 90 percent of the area between 290 and 400 nm. In the past a few years, FDA became increasingly concerned about that fact that too much exposure to long wavelength UVA may cause skin cancer in addition to skin-aging and allergies. Therefore, the FDA

has proposed the addition of a more stringent requirement: a UVA-I/UV ratio of 0.7 or higher. This ratio is the ratio of the average absorbance in UVA-I (340 - 400 nm) to that of the total UV (290 - 400 nm).

European sunscreens must have balanced protection: A UVA protection factor (*in-vitro* or *in-vivo*) of one third of the *in-vivo* SPF and a critical wavelength of at least 370 nm. The broad spectrum UV protection requires that if your formula's *in-vivo* PA (UVA) measurement is a 10, the label claim for SPF cannot exceed SPF 30.

Lebelled category	Labelled sun protection fator	Measured sun protection factor (measured in accordance with the principles recommended in point 10 (a))	Recommended minimum UVA protection factor (measured in accordance with the principles recommended in point 10 (b))	Recommended minimum critical wavelength (measured in accordance with the principles recommended in point 10(a))
Low protection	'6'	6-9.9	1/3 of labelled sun protection factor	370 nm
	'10'	10-14.9		
Medium protection	'15'	15-19,9		
	'20'	20-24.9		
	'25'	25-29.9		
High protection	'30'	30-49.9		
	'50'	50-59.9		
Very high protection	'50+'	60 ≤		

Table 2: Regulatory framework for SPF and UVA claims in the EU

On the current marketplace, there are SPF 30 and 50 sunscreen formulas claiming ZnO as the sole sunscreen active **(Table 2)**. These formulas typically contain boosters like antioxidants, anti-inflammatories, anti-irritants, film formers, or UV absorbing emollients like butyl octyl salicylate to achieve high protection.

There are detailed regulations, specifically in the EU, regarding the use of nanomaterials, including mandatory annual declaration in France, formula registration and notification, and the labeling of finished goods. European Union regulators generally define the particle size of a nanomaterial by its internal structures and, consequently, any grades of mineral UV filters are in the nano size range if determined by measuring the shortest dimension, 50 percent of the particles have a particle size below 100 nm by number weighted size distribution. For products destined for the EU market, very specific criteria for nanomaterials, including a very limited list of allowed surface treatments, must be observed. The formulator must decide how to measure and determine nanomaterials, as there are two interpretations: by the primary particle size or by the aggregate size as interpreted by Cosmetics Europe.

The use of TiO_2 and ZnO also has some restrictions due to their toxicity profile. TiO_2 is considered to be carcinogenic by the EU commission and the state of California if used as airborne, unbound particles of respirable size [7,8]. As a result, nano TiO_2 cannot be used in products that may lead to exposure of the end-user's lung by inhalation. No TiO_2, nano or non-nano, can be used in California if it can be airborne and respirable. As for ZnO, SCCS has found that it has local effect in human lungs. Therefore, neither nano nor non-nano ZnO are allowed in a product that may lead to exposure of the end-user's lung by inhalation [9] In practice, the respirable particle is defined as a particle with an aerodynamic particle size under 10 µm. Fortunately, most TiO_2 and ZnO powders have an aerodynamic particle size over 10 µm because of the aggregation and/or agglomeration. Even so, it is highly recommended to test their aerodynamic sizes in the form of delivery of the formulation if the formulation can be airborne like powders or sprays.

From consumers desires to personal care formulations

In 2021, more than half of consumers worldwide consider themselves regular users of sunscreen. However, only 22% apply sunscreen daily, and 37% use sunscreen regardless of the activities they plan for the day when it is sunny outside. The first figure is subject to wide regional variations. In Europe, less than 10% of consumers protect their skin from UV radiation daily [2].

Consumer expectations and the changing attitude of cosmetic manufacturers towards sustainable beauty products also affect sunscreen products. The development of a new sunscreen product requires the consideration of several aspects.

In addition to continuous awareness of the health risks of UVA and UVB light, a pleasant skin sensation during and after use is one of the most important factors to increase the frequency of sunscreen use. Consumer acceptance is critical to a successful sunscreen product.

A satisfactory experience prompts the consumer to use the right amount of product to achieve the desired protection, to reapply the product, and ultimately to likely consider purchasing the product again.

In addition to the focus on environmental aspects and sustainability, one of the most important parameters for high consumer acceptance of a product is sensory performance or, in short, skin feel. Multifunctional products such as "skin protectants": hybrid sunscreens with a skin care-oriented approach (ingredients, claims, formulations) could be the solution to convince consumers who do not yet use mineral UV protection products **(Figure 2)**. Surprisingly, awareness of mineral filters is still low among the general population today. However, there is a growing interest in natural, skin-friendly, and environmentally friendly sunscreens, which will lead to an increase in mineral sunscreen products.

W/O Sun Lotion SPF 30*

In-vitro UVA-PF**	17,2
Critical Wavelength	374 nm

PHASE	MATERIAL NAME	INCI	%	SUPPLIER
A	Dehymuls® PGPH	POLYGLYCERYL-2 DIPOLYHYDROXYSTEARATE	5.00	BASF
	Monomuls® 90-O 18	GLYCERYL OLEATE	3.00	BASF
	SymMollient® PDCC (102119)	PROPANEDIOL DICAPRYLATE/CAPRATE	**7.00**	**SYMRISE**
	Caprylic/Capric Triglyceride	CAPRYLIC/CAPRIC TRIGLYCERIDE	5.00	/
	PCL Liquid® 100 (660089)	CETEARYL ETHYLHEXANOATE	**8.00**	**SYMRISE**
	SymDecanox® HA (972276)	CAPRYLIC/CAPRIC TRIGLYCERIDE, HYDROXYMETHOXYPHENYL DECANONE	0.50	**SYMRISE**
	Dispersun DSP-OL 100	POLYHYDROXYSTEARIC ACID	1.00	INNOSPEC
	Thixcin® R PC	TRIHYDROXYSTEARIN	0.50	ELEMENTIS
B	**Neo Heliopan® ZnO 40 (102569)**	ZINC OXIDE [NANO], TRIETHOXYCAPRYLYLSILANE	**26.31**	**SYMRISE**
C	Water	AQUA	33.49	/
	Neo Heliopan® Hydro (103089)	PHENYLBENZIMIDAZOLE SULFONIC ACID	**1.00**	**SYMRISE**
	Neo Heliopan® AP (106796)	DISODIUM PHENYL DIBENZIMIDAZOLE TETRASULFONATE	**2.00**	**SYMRISE**
	Glycerin	GLYCERIN	3.00	/
	Magnesium Sulfate Heptahydrate	MAGNESIUM SULPHATE HEPTA HYDRATE	0.80	MERCK
	Sodium Gluconate	SODIUM GLUCONATE	0.10	JUNGBUNZLAUER
	Biotive® L-Arginine (621277)	ARGININE	**1.50**	**SYMRISE**
	Phenoxyethanol	PHENOXYETHANOL	**0.90**	**/**
	SymSave® H (979940)	HYDROXYACETOPHENONE	**0.50**	**SYMRISE**
	Hydrolex™ E (379434)	ETHYLHEXYLGLYCERIN	**0.10**	**SYMRISE**
D	**Fragrance SUN Beauty (482606)**	FRAGRANCE	**0.30**	**SYMRISE**

Manufacturing Instructions

Phase A:
Mix all ingredients of phase A and heat up to 85°C.

Phase B:
Add phase B to phase A and homogenize with an UltraTurrax® T25 for 10 min at 10000 rpm.

Phase C:
Heat up phase C to 75°C.
Add phase C slowly while stirring to phase A/B.
Homogenize phase A/B/C (UltraTurrax® T25 / 13000 rpm / 3min)
Cool down to ambient temperature while stirring.

Phase D:
Add phase D and homogenize again (UltraTurrax® T25 / 13000 rpm / 1.5min)

pH (water phase, 23°C) > 7.0
Viscosity: 7292 cp
(Brookfield DVIII CP51/10RPM/20°C)

Preservative efficacy test
Ph. Eur.: Crit. A

* tested according to the ISO 24444:2019 "*In vivo* determination of Sun Protection factor (SPF)" on a 5-subject panel test, the formula has an ***in vivo* SPF mean 34.3**.
** Measured according to the ISO 24443:2012 "Determination of UVA photoprotection *in vitro*" the emulsion has an ***in-vitro* UVA-PF 17,2**. (Used plates: PMMA WW5 from Schoenberg)

Fig. 2 Formulation of a hybrid sunscreen - W/O Sun Lotion SPF 30* for Sport

Current users have a positive perception of the protective effect, the care effect, and the gentle ingredients, and tend to judge the white cast when spreading mineral sunscreens as not annoying. Non-users are considering buying mineral sunscreens in the future, but the main obstacle so far has been product characteristics: parameters such as difficult spreadability, whitening, and rich skin feel. To convince non-users, consumers need to be better educated about mineral UV filters and formulas with excellent skin feel and minimal whitening effect. Consumers are likely to use mineral sunscreens in more frequent demand in the future because the positive effects, such as suitability for all skin types, including sensitive skin, and more compatible with the environment, outweigh the negative effects.

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authors

Yun Shao and David Schlossman
Kobo Products, Inc | www.koboproductsinc.com

Marek Busch and Ev Suess
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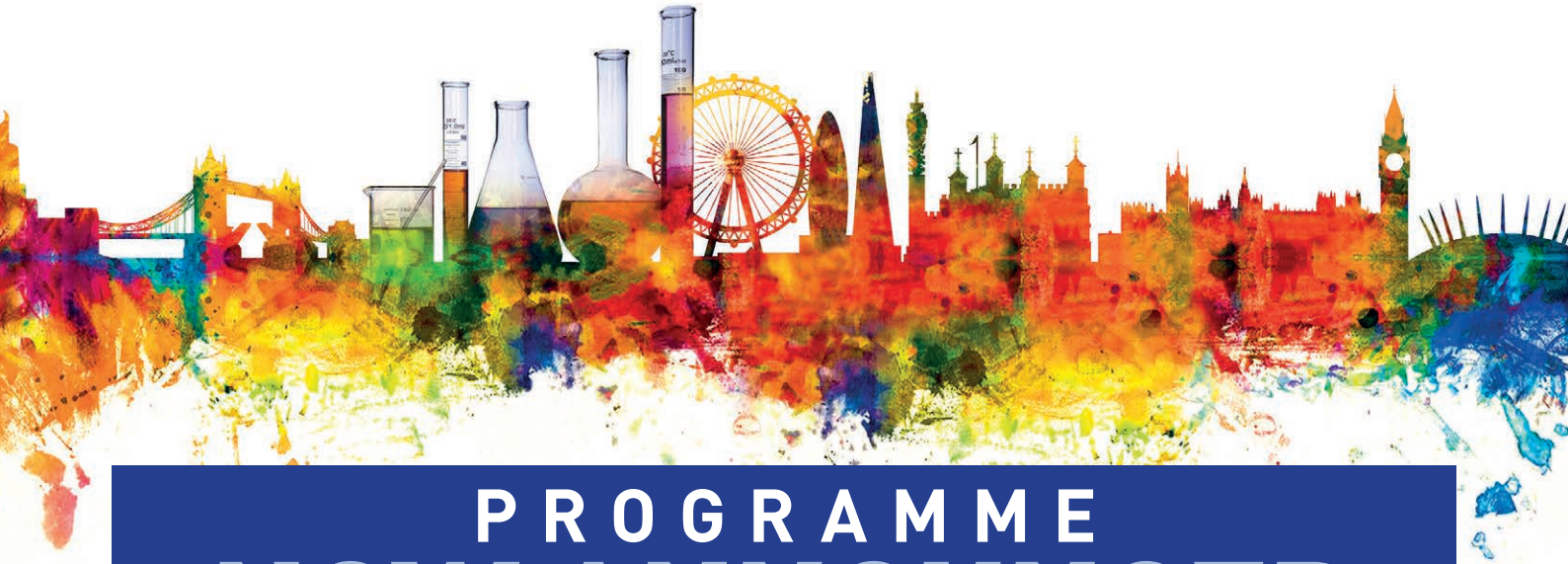

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Solid Sun Care

N. Schiemann, A-S. Gardes

Solid Product Forms as an Emerging Trend

When we analyse new product launches a significant part is about solid product forms [1].
In this context solid bar formats are the natural place to start. A much more concentrated formulation in terms of ingredients, solid formats can have a lower cost per use than traditional formats, which contain a lot more water, particularly in the haircare and bath or shower categories.
Packaging of solid bars can provide further sustainable benefits, using biodegradable cardboard as opposed to the large plastic container required to house liquid shampoo.
Mintel [1] recommends that brands think more broadly when entering the waterless category than just eliminating water from the product. The true impact will come from how that product is used by the consumer, and more importantly, what is left over after the product is used up.
Boosting a solid product form works very well with oil soluble ingredients and particles. However, these ingredients should support the skin barrier and not compromise the skin microbiome.
Based on a product concept, which has been developed at the IMCD Skin Care and Sun Care Center of Excellence in Paris [2] and which has been awarded by the SEPAWA Innovation Award 2021 [3] we find a lot of inspiration.

Considerations for Sun Care

Several cosmetics experts share the opinion, that the least impact cosmetics can have on the natural microbiome of the skin, is the best. Especially when it comes to applications which imply a long contact with the skin and focus on protection against environmental impacts, like UV rays, the natural microbiome should not be compromised too much. There are no studies available yet, which demonstrate this statement. It is just logical, that least impact is the best, which is in line with the spirit of the age, that nature works best and the least impact on natural balance is the goal.

We might conclude that Sun Care Products should support the natural functions of the skin at best, but not harm the functions of the skin. In this context we should be always aware that the microbiome is an essential part of the skin barrier function.

Beauty Salon in a Pill Box – Finger Beauty

The formula design comprises biodegradable ingredients derived from nature, and which can be stored in boxes instead of jars **(Figure 1)**. A balanced ratio of waxes, emollients, water and actives together with a water in oil emulsifier blend is important to provide a great flexibility in applications, which are not limited to skin care only. Even applications for hair care and sun care have been created based on this concept.
Although there is a big part of so-called waterless products, in many circumstances it is important to have a given amount of water in the product, since there are many water-soluble active ingredients which are beneficial in Beauty Care. And even more, water adds natural moisturization for immediate effect and imparts lighter sensorial properties compared to 100% oil-based formulations.
Trials with different combinations of waxes, lipids, and watery phases together with a complex emulsifier resulted in

Fig. 1 Beauty Salon in a Pill Box – prototypes

successful combinations of beads, which can be modified according to their needs. The balance between the selected waxes and oil components is crucial in obtaining the desired ball to cream beads.

The given level of water is sufficient to still contain effective water-soluble actives and provide some moisture and light product feel, especially when thinking about hair applications, which are possible based on the same frame formula, which shows primarily applications in skin care.

It is possible to add various colors and functional particles, as well as functional actives and a broad variety of water and oil soluble actives. Due to its solid state the beads can effectively take loads of functional particles, which easily boost solid forms.

Mineral sunscreens can be the above-mentioned functional particles. Based on this idea a Solid Sun Cream **(Figure 2)** with hydrating effects has been formulated [4].

Fig. 2 Solid Sun Care Beads – Hydra Sunpearls by IMCD

Phase	INCI	Weight %		ISO*
A	Polyglyceryl-6 Polyricinoleate, Polyglyceryl-2 Isostearate, Disteardimonium Hectorite	1.00	Emulsifier	0.97
	Heptyl Undecylenate	13.00	Emollient	1.00
	Triheptanoine	16.95	Emollient	1.00
	Silica	3.00	Functional Powder	1.00
	Polyhydroxystearic Acid	0.45	Additive	1.00
	Zinc Oxide, Coco-Caprylate/ Caprate, Polyhydroxystearic Acid	18.00	UV Sunscreen	1.00
B	Oryza Sativa (Rice) Bran Wax	8.00	Thickener & Rheology Modifier	1.00
C	Water	33.00	-	1.00
	Various water-soluble actives	1.00	Active	0.98-1.00
	Propanediol	5.00	Humectant	1.00
	Caprylyl Glycol, Benzyl Alcohol	0.60	Preservative	0.00
		100.00		**99.35**

* The formulation is in line with clean beauty concepts and ISO 16128 standard [5].

Formulation 1: Solid Sun Cream with hydrating effects

Polyglyceryl-6 Polyricinoletate, Polyglyceryl-2 Isostearate, Disteardimonium Hectorite, available as NIKKOL Nikkomulese WO-NS from NIKKOL Group is a key ingredient [6]. This emulsifier forms a W/O emulsion and contributes to a light texture and an optimized glide, thanks to the thixotropy provided by the modified hectorite. It is known for its application in Sun Care formulation containing mineral sunscreens.

Heptyl Undecylenate, i.e. LexFeel Natural™ from INOLEX [7] is a 100% natural cyclomethicone alternative with a light and ultra-dry skin feel. Also, enhances dispersing and spreading characteristics of the formula.

Each bead melts in the palm of the hand and turns into a light and non-greasy cream, thanks to Triheptanoin a 100% naturally derived emollient, which can be purchased as SustOleo™ MCT from INOLEX. [8].

Rice Bran Wax or other suitable ingredients, like Ucuuba Butter from Jan Dekker [9] or Sunflower Wax are necessary to solidify the formula and allow to keep the beads form while offering a good product feel. The balance between waxes and oils determines the melting effect.

Zinc Oxide can be added as a powder, e.g. UV Protec from Grillo [10], which needs to be predispersed in suitable solvents with dispersing aids like Polyhydroxystearic Acid.

Easier is the use of readily available premixes, e.g. UV CUT ZnO-65-CC from Grant Inc., which is a dispersion of zinc oxide in coco-caprylate/caprate and polyhydroxystearic acid. This nature-derived UV sunscreen is being used in COSMOS formulas. It is light, non-sticky and does not whiten the skin [11].

Oil soluble actives as well as water soluble ones can be added, but also functional particles.

Depending on selection of actives and preservative system, a naturality of 1 (100%) is possible

The application is very versatile, since the concept can be transferred to skin care with all aspects like facial and neck applications, hand and feet care, as well as to hair care (conditioning, treatments), but also to cleansing.

To realize the concept a hot process at up to 80°C is necessary. This can be a challenge for liquid product manufacturers, but for everybody making classic emulsions the process can be easily adapted. Additional requirement is the hot fill at 70-75°C.

Based on experience two beads with 5-10 mm diameter offer the ideal dose for a good hydration and protection of the area around the nose, the eyes or your hands. Each bead melts on the fingers or in the palm of the hand and turns into a light and protective cream.

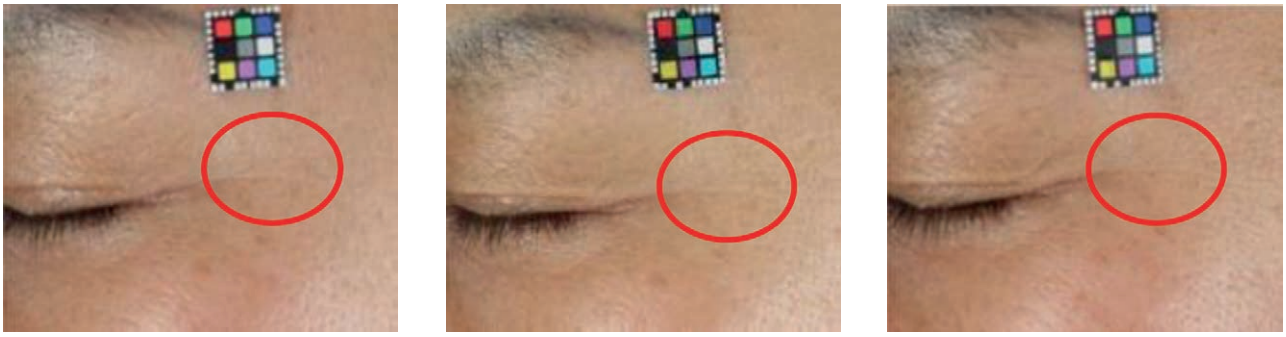

Fig. 3 Improvement of skin appearance over 8 weeks due to application of 0.1% HAbooster

Boosting ingredients

Beside a higher SPF by means of increasing the ZnO concentration, we might consider SPF-boosters, like Silica Beads, available e.g. as Sunspheres H-52 from AGC [12] but also ingredients which provide some additional moisturizing benefits, e.g. HAbooster, a Hyaluronic Acid, effective at low concentration [13]. This Hyaluronic Acid with a very low molecular weight, which can penetrate to lower layers of the skin, has been tested *in vitro*, by a human patch test and repeat insult patch test as non-irritant.

Even at a very low concentration of 0.1% it improves skin texture and appearance over time **(Figure 3)**.

In clinical studies an increase in collagen production, skin moisture, as well as an improvement of skin texture could be substantiated.

In a clinical study the effective reduction of wrinkles (crow's feet and nasolabial folds) by using a lotion containing 0.1% HA-Booster over a period of 4 to 8 weeks could be proven. Even a reduction in sagging in the cheek area has been observed **(Figure 4)**.

Beside these ingredients we should have a look to oil soluble actives, which are beneficial for sensitive skin.

Particles with proven efficacy in improving and regenerating the skin barrier, like Kaolin-Types from Terramater [14] or naturally derived Fullerene from VC60 (15) can further boost the effects of this application, without compromising the skin microbiome.

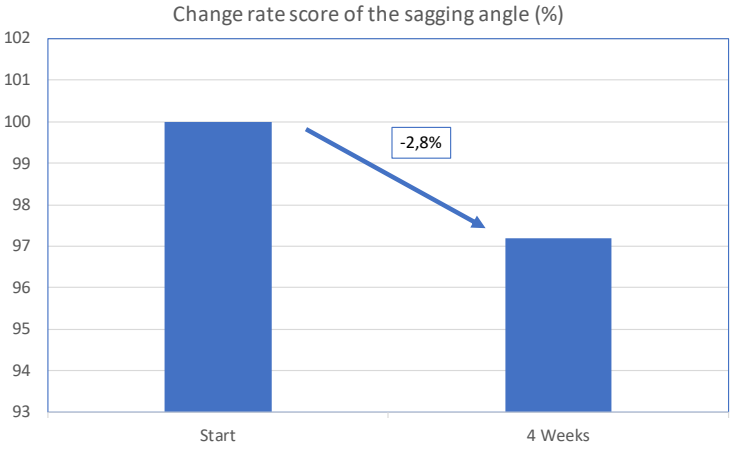

Fig. 4 Change in sagging, tested by Moire Topography

Conclusion

The shown application of a solid sun cream, which can be applied on discrete parts of the skin provides possibilities to have the least impact on the skin microbiome but also helps to regenerate the skin and its barrier function with natural ingredients, especially functional particles providing UV protection. These benefits are also coming with a great ease of use and an amazing silky and both comfortable sensorial properties.

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[10] UV Protec from Grillo
[11] UV Cut from Grant INC.
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[13] HABooster from Kewpie
[14] TERSIL GEM from TERRAMATER
[15] LipoFullerene N® VC-60.

authors


Nora Schiemann


Anne-Sophie Gardes

Dr Nora Schiemann | Regional Technical Manager DACH & BNL, Cologne
Anne-Sophie Gardes | Technical Director EMEA, Paris
IMCD, Business Group Beauty & Personal Care
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Bright Partner for Bright Skin

A. Gafari, J. Hans, M. Lochouarn

abstract

As a pioneer explorer of the exposome, Symrise is constantly developing new solutions for pigmentation. Dark spots are now perceived as the new wrinkles, and Symrise has been exploring the paths to tackle this specific concern and looking for a bright partner with optimized efficacy.
To capitalize on our blockbuster SymBright® 2036, we looked for a smart & efficient combination! Niacinamide – vitamin B3 – appeared as a perfect match to enhance the efficacy of SymBright® 2036 thanks to its multiple virtues leading to the development of SymBright® PLUS, a ready-to-use liquid blend.

Testing efficacy of SymBright® PLUS

UV light can induce melanogenesis in the skin through two major distinct pathways: by direct activation of the melanocyte or via an indirect pathway by generating Reactive Oxygen Species (ROS) e.g. in surrounding keratinocytes. These keratinocytes react to ROS by producing stress signals which are then secreted and subsequently stimulate melanogenesis.
The newly launched ingredient SymBright® PLUS has been developed as a skin brightener. Therefore, we evaluated its efficacy on human skin explants exposed to UV irradiation. As the generation of ROS as starting point for the indirect activation pathway is a very quick process, we studied the capability of SymBright® PLUS to reduce the amount of ROS in skin explants irradiated with 60 J/cm² UVA.

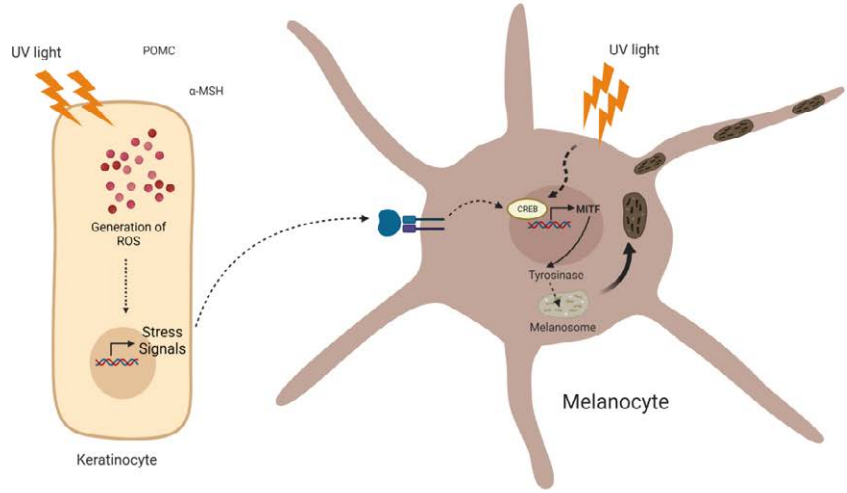

Fig. 1 Schematic representation of direct and indirect route for melanogenesis induction by UV light.

In **Figure 2**, cross sections of skin explants are displayed with a green fluorescence signal indicative of the presence of ROS in the tissue.

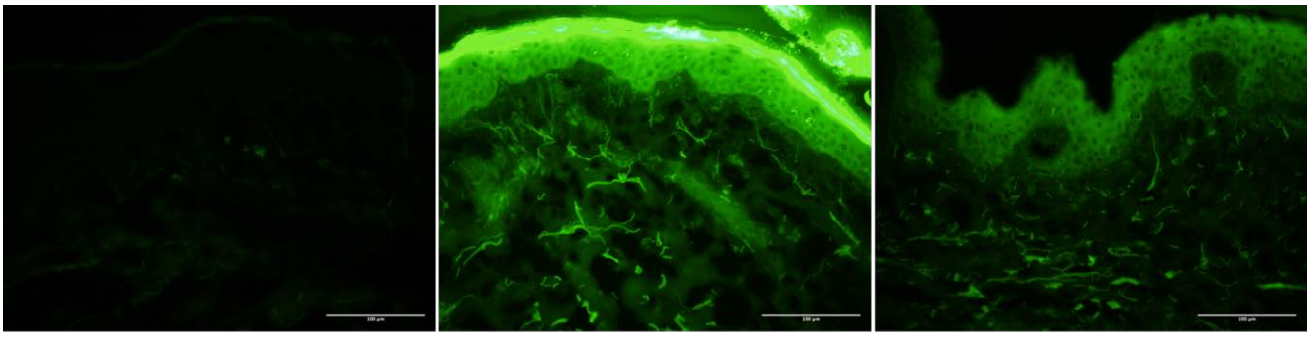

Fig. 2 Fluorescence staining (DCFH-DA) of histological cross sections from human skin explants (female donor, age 51, ITA angle 36° "intermediate"); intensity of fluorescence is correlated to the presence of ROS in the tissue. Scale bars represent 100 µm.

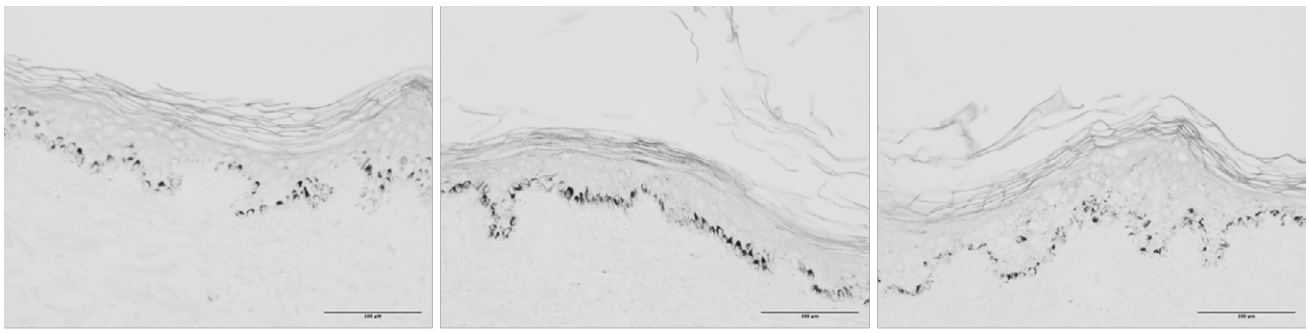

Fig. 3 Assessment of melanin content by Fontana Masson staining in human skin explants after 7d of treatment with the test items and subsequent exposure to 75 mJ/cm2 of UV B irradiation. The black staining is indicative of the presence of melanin and was quantified by image analysis. Scale bars represent 100 µm.

Quantification of the fluorescence allows the evaluation of the ROS reducing activity of the test samples. **SymBright® PLUS led to a 66% decrease in ROS score** vs UV-irradiated control. Also tested were the components making up SymBright® PLUS in their respective concentrations, and none of the single ingredients reached the efficacy in ROS reduction of SymBright® PLUS. This result demonstrates the superiority of SymBright® PLUS vs its single components in terms of ROS reduction in the human skin.

In a second experiment, we analyzed the effect of SymBright® PLUS on UV-induced pigmentation. Human skin explants (female donor, age 43, ITA angle 24° "tanned") were exposed to UVB irradiation daily for 7 days with a dose of 75 mJ/cm^2. Quantitative evaluation of melanin content in the skin explants was performed using Fontana-Masson staining. The skin explants where SymBright® PLUS was applied showed a **26% reduction of melanin** vs the UV control (see **Figure 3**).

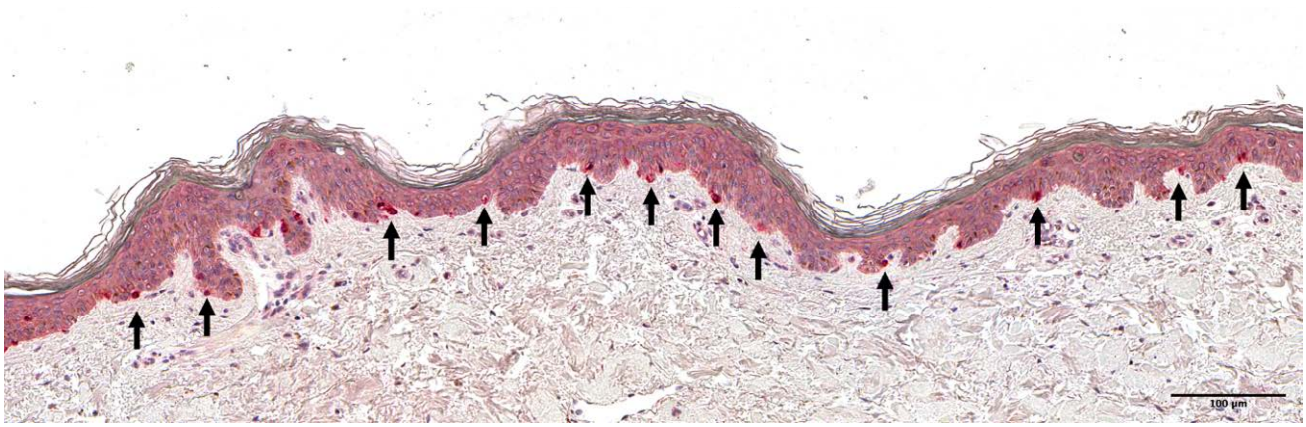

Fig. 4 Representative image of Melan-A signals in cross sections of human skin explants after 7 d treatment with test items and daily irradiation with 75 mJ/cm2 UVB. Image shown is vehicle control with UVB irradiation. Cells colored in deep red (examples shown by black arrows) contain Melan-A and therefore are considered to be engaged in de novo melanogenesis. Scale bar represents 100 µm.

Changes in melanin content can be caused by degradation of existing melanin or by reduction of the biosynthesis of the pigment. To further assess the action on melanogenesis, we analyzed the abundance of Melan-A as marker of de novo formation and maturation of melanosomes, the organelles in which melanogenesis takes place.

By comparing UVB-irradiated skin explants we identified **a 64% reduction of Melan-A signals** when SymBright® PLUS was used vs vehicle control. Also, we noticed that SymBright® PLUS was able to outperform its single compounds in the same experimental setup.

Taken together our findings indicate a very good reduction of UV-induced ROS species in human skin explants, as well as the ability to help regulate melanogenesis, potentially exerting an effect both on the direct and the indirect pathway for UV-induced melanogenesis. The product outperforms its single components in terms of ROS inhibition and de novo melanogenesis, clearly demonstrating the added value of the product.

Conclusion

SymBright® PLUS is a versatile and – *in vivo* proven - efficient solution for the prevention of uneven skin pigmentation and hyperpigmented spots. Thanks to its easy-to-use liquid form, it can be used in multiple applications such as everyday face & body care, anti-spot treatments, tissue masks and more.

authors

Amélie Gafari | Global Product Manager,
Dr. Joachim Hans | Molecular & Cell Biology Manager,
Marine Lochouarn | Marketing Manager

Symrise Cosmetic Ingredients Division
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Irritated, Itchy, Scaly, Seborrheic Scalp:

Causes and Relief with a Proprietary, Cold-Pressed *Nigella sativa* (Black Seed) Oil Standardized to 3% Thymoquinone

L. von Oppen-Bezalel, J. S. Jurenka

abstract

Medicinal herbs have been used as natural healing and cosmetic remedies since ancient times. The annual flowering plant, *Nigella sativa*, its black seeds and oil derived from them were used for medicinal and beauty routines since the ancient Egyptian pharaohs and queens. It has been studied more recently for its many health and cosmetic, anti-inflammatory, antioxidant, and antifungal benefits. To harness the power of the *Nigella sativa* (black seed) oil (BSO), its active constituent, thymoquinone, and their demonstrated benefits, a patent pending, cold-pressed, full-spectrum black seed oil, standardized to 3% thymoquinone and very low FFAs was developed.

Branded as B'utyQuin™ for cosmetic use, BSO has been studied *in vitro* to determine the mechanisms responsible for skin disorders such as, irritated, itchy, scaly skin and scalp and seborrheic dermatitis. The studies included BSO's antimicrobial effects on microorganisms such as *Malassezia furfur*, *Candida albicans* and *Staphylococcus aureus*, often associated with such disorders. This has been followed by clinical research to establish efficacy, safety and compatibility of BSO as a topical active ingredient for the scalp. BSO's ability to positively impact the irritated, itchy, scaly scalp associated with seborrheic dermatitis has been revealed. In a blinded, controlled clinical trial in subjects with mild to moderate seborrheic dermatitis, topical application of scalp serum with 5% B'utyQuin for 28 days resulted in significant improvements in scalp oiliness, irritation, itchiness, scaling, and overall health of the scalp as compared to baseline.

Introduction

The annual flowering plant, *Nigella sativa* (*N. sativa*, family Ranunculaceae), is native to southeastern Europe, western Asia, the Middle East, and northern Africa and oil from its black seeds, black seed oil (BSO), has been used for medicinal and beauty treatments for centuries [1,2]. More recently, black seed oil has been studied for its many health and dermatologic, anti-inflammatory, antioxidant, and antifungal benefits [3-7]. Determination of *N. sativa*'s primary constituent, thymoquinone (TQ), has resulted in a growing interest in its pharmacological activities, including its powerful antioxidant, antimicrobial and anti-inflammatory properties [6-14].

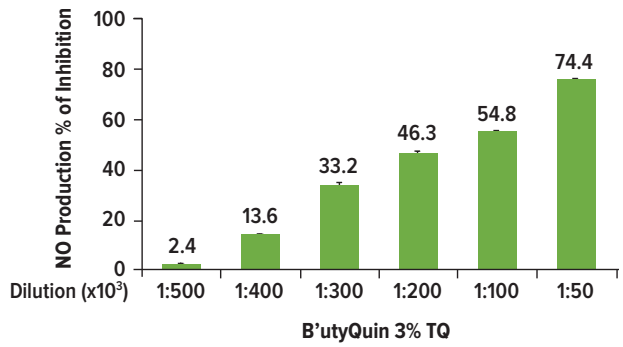

Fig. 1 Anti-inflammatory Performance of BSO with 3% TQ and 1.8% FFA

To harness the incredible power of thymoquinone, TriNutra™ Ltd., has cultivated a full-spectrum, cold-pressed BSO standardized to 3% thymoquinone and very low free fatty acids, the highest quality and most potent thymoquinone concentration available on the market. This farm-to-finished black seed oil features full sustainability and traceability by ensuring that farm history, cultivation of varietals, growing and harvesting conditions, seed storage, extraction, and processing techniques are completely controlled in one location to guarantee a superior quality material. TriNutra's cold-pressed black seed oil is branded as B'utyQuin™ for dermatologic use and its constituents act synergistically to maximize the mitochondrial functions, metabolism, energy (ATP) production as well as anti-inflammatory **(Figure 1)**, antioxidant, and antimicrobial effects of the oil. Based on the demonstrated mechanisms and beneficial properties of this standardized BSO, it was hypothesized that it may be effective in modulating itchy, scaly, irritated skin and scalp, associated with dandruff and seborrheic dermatitis.

Seborrheic Dermatitis

Seborrheic dermatitis (SD) of the scalp is a common skin condition whose incidence in adults peaks between 40 to 60 years of age [15] and can be a chronic or relapsing condition. SD is characterized by irritated, erythematous patches,

with flaky, oily or dry scales in sebum-rich areas, primarily on the scalp and face. SD is a multifactorial condition that can involve several predisposing factors. Systemic inflammation, harsh hair care products, an inflammatory or allergenic diet, and an imbalance in the skin microbiome are all thought to contribute to the occurrence of seborrheic dermatitis [16-19]. The presence of some or all of these factors can lead to a disruption of the skin microbiome and increased levels of the opportunistic microorganisms *Malassezia furfur*, *Candida albicans*, and *S. aureus*. It has been established that *M. furfur* has a significant correlation with the severity of seborrheic dermatitis, dandruff and psoriasis [20].

Black Seed Oil – *In vitro* Research

BSO may have the potential to inhibit overgrowth of certain fungi and bacteria and support maintenance of a healthy skin and gut microbiome. Several studies have demonstrated antimicrobial activities by black seed oil [21,22]. *In vitro* research demonstrates the importance of standardization of cold-pressed black seed oil in achieving this biological effect, primarily through standardization of the level and the ratio of thymoquinone (TQ) and free fatty acids (FFA).

Generally, the thymoquinone content in *N. sativa* seeds ranges from concentrations of 0.3% to 1.0% (w/w), with differences observed between seeds obtained from different countries and regions [23]. Cold-pressed oil obtained from *N. sativa* seeds predictably contains on average TQ concentration in the range of 0.5% to 1.5% (w/w). Various procedures have been developed for obtaining TQ-rich fractions from *N. sativa* seeds, however, they are often complex, expensive, and selective for enriching TQ levels at the expense of losing other important oil components [24,25].

The manufacturing process used for the preparation of the oil in the current study is a cold-press, environmentally friendly, physical process that does not require the use of organic solvents or supercritical CO_2. A cold-pressed oil maintains the inherent composition of the oil as well as the purity, allowing consistent delivery of a full-spectrum oil with optimal strength and potency. **Table 1** summarizes the different BSOs used to study the best BSO composition for antimicrobial benefits.

FORMULATION	Thymoquinone (TQ) %	P-cymene %	Free Fatty Acids (FFA) %
Black Seed Oil 3% TQ Low FFA	3.21	1.33	1.80
Black Seed Oil 3% TQ high FFA	3.08	1.21	10.30
Black Seed Oil 0.5% TQ Low FFA	0.53	0.21	2.00
Black Seed Oil 0.5% TQ High FFA	0.42	0.23	9.80

Table. 1 BSO Compositions for Antimicrobial Benefits

Four oils with either high or low TQ content (estimated 3% or 0.5%, respectively), and low or high FFA content (2% or 10%, respectively), were assessed for their antifungal qualities. A disc diffusion assay assessed the ability of *N. sativa* oil to inhibit *M. furfur* growth. Three of the oils (100% oil) inhibited the growth of *M. furfur* in a TQ and FFA dose-dependent manner, except the oil composed of 0.5% TQ and 10% FFA which did not inhibit any growth. The oils containing 3% TQ showed the most potent inhibition of *M. furfur* growth, with superior

antifungal potency for the one with low FFA (<2%). This study shows that the single component, TQ was the most significant factor affecting inhibition of *M. furfur* growth, an efficacy that declines in the presence of high level of FFAs **(Figure 2)**. This observation was strengthened by similar results while evaluating growth inhibition of Fluconazole-resistant and sensitive *C. albicans* strains. As with *M. furfur*, the BSO containing 3% TQ and low 2% FFA exhibiting the strongest inhibition effect against both *C. albicans* strains, suggesting antifungal activity is dependent on the concentration of TQ and that a low level of FFA is correlated with maximum efficacy [22].

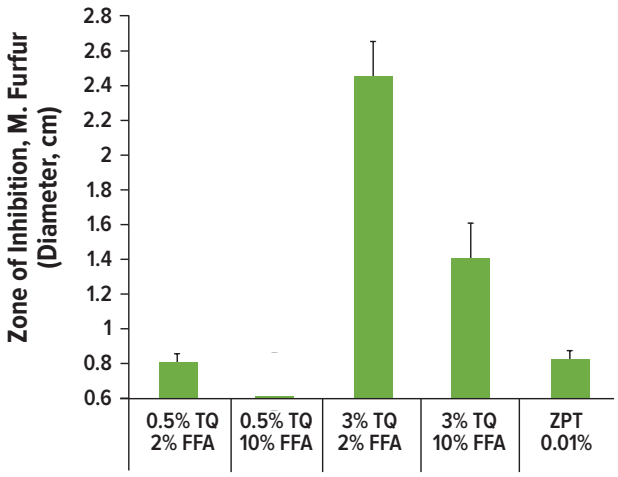

Fig. 2 Antifungal Activity of BSO on *Malassezia furfur*

Evaluation of the antibacterial properties of *N. sativa* oils painted a different picture. *N. sativa* oils strongly inhibited the growth of Methicillin-resistant *S. aureus* (MRSA); however, no preference was observed for different levels of TQ or FFAs. The fact that all *N. sativa* oils tested inhibited the growth of *S. aureus* supports the anti-bacterial benefits of the oils, but further research is needed to investigate which oil constituents are functional to optimize this antibacterial benefit [22].

Collectively, these findings are confirmation that the antimicrobial activity of Trinutra's unique BSO with 3% TQ and low FFA composition (B'utyQuin) has a significant role in the efficacy of the oil's antifungal (*M. furfur* and *C. albicans*) and anti-bacterial (*S. aureus*) activities, including against antibiotic-resistant strains. Thus, regulating the growth of these opportunistic microorganisms can play an important role in maintaining homeostasis of the skin microbiome and potentially inhibiting the development of SD [22].

The mechanisms of action attributed to the observed improvements in seborrheic dermatitis manifestations are likely a result of synergy between the previously established antimicrobial, anti-inflammatory, antioxidant, and enhanced mitochondrial energetics properties of B'utyQuin's 3% TQ and low free fatty acid composition **(Figure 3)**.

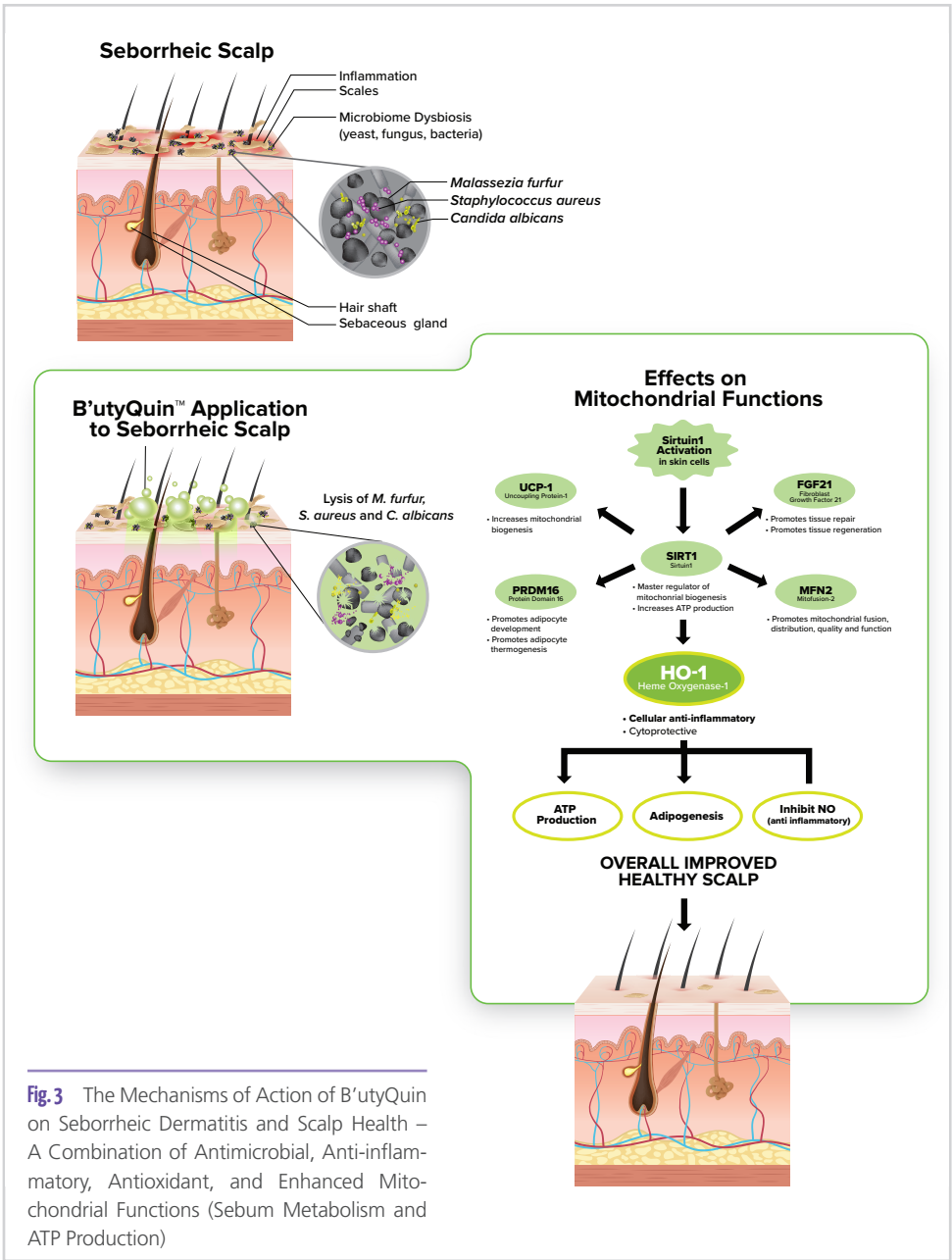

Fig. 3 The Mechanisms of Action of B'utyQuin on Seborrheic Dermatitis and Scalp Health – A Combination of Antimicrobial, Anti-inflammatory, Antioxidant, and Enhanced Mitochondrial Functions (Sebum Metabolism and ATP Production)

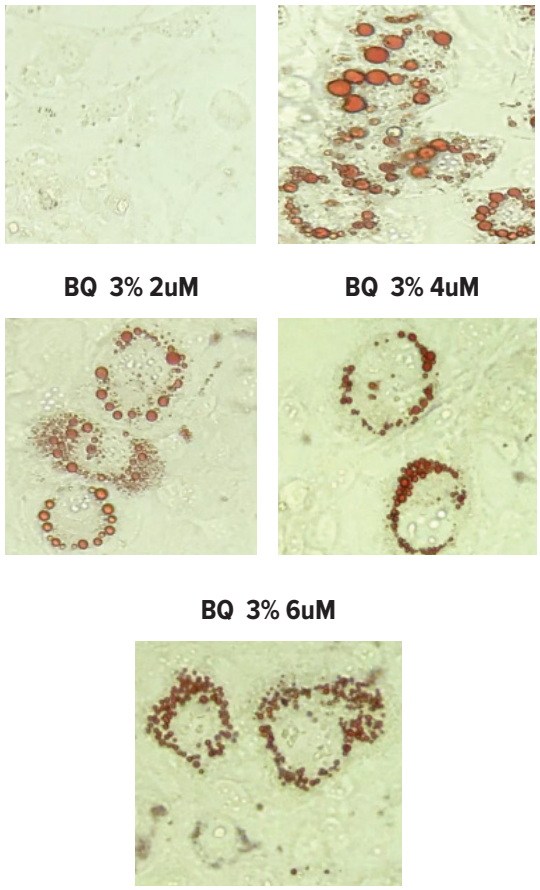

Fig. 4 B'utyQuin Effect on Adipogenesis and Adipocytes Size.
*Effect of BSO with 3% thymoquinone oil on oil droplets formation in 3T3 adipocytes showed a significant reduction of lipid droplets formation in 3T3 adipocytes at day 6. (n=4), *#p<0.05* vs. control.

B'utyQuin (BSO) was not only effective at significantly inhibiting growth of two different fungal organisms (*Malassezia furfur* and *Candida albicans*) but was also effective against resistant strains of *S. aureus*, three opportunistic pathogens associated with seborrhea and other disorders of the skin and scalp. The anti-inflammatory action of BSO is well established **(Figure 1)**

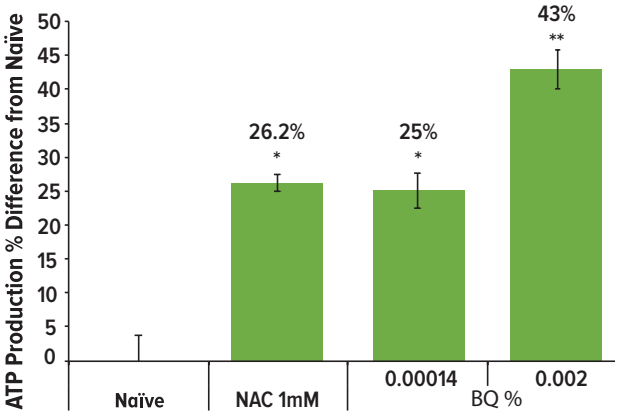

Fig. 6 B'utyQuin effect on ATP Production.
The effect of B'utyQuin on production of ATP in Keratinocytes (HaCaT cells) compare to baseline (Naïve cells) and positive control (N-Acetyl-cysteine (NAC) 1mM)

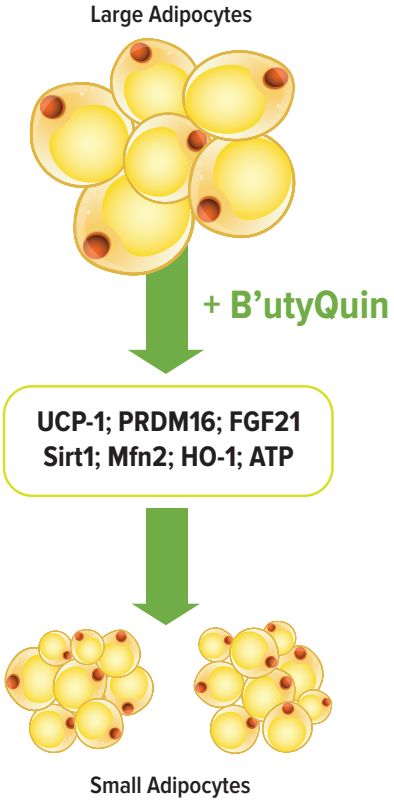

Fig. 5 B'utyQuin Effect on Key Regulators of Mitochondrial Biogenesis in 3T3-L1 Adipocytes.

and is thought to be responsible for improvements in scalp redness and irritation. The established antioxidant property of BSO is likely responsible for modulation of oxidative stress and down-regulation of pro-inflammatory molecules that may be at play in the occurrence of SD. BSO's significant effect on adipogenesis and lipid droplet size in adipocytes **(Figure 4)**, upregulation of mitochondria biogenesis, and conversion of white to thermogenic beige adipocytes **(Figure 5)** may be responsible for BSO's ability to decrease the scalp oiliness and excess sebum formation associated with seborrheic dermatitis. Increased mitochondrial ATP production **(Figure 6)** [unpublished data of TriNutra] and anti-inflammatory mechanisms are likely contributing to the BSO's soothing and calming effects reported by the clinical study subjects [26].

Clinical Research Study

This blinded, controlled, single-center 28-day study was conducted to verify the efficacy, compatibility and safety of a cosmetic product with 5% B'utyQuin (Scalp Serum), containing a standardized cold-pressed black seed oil with 3% thymoquinone and 2% free fatty acids and to support the results of previous *in vitro* research. The study involved ten male and female subjects, aged 18-65 years old (all skin types), with mild to moderate seborrhea of the scalp. Evaluation of changes in scalp condition, erythema, itchiness, oiliness, scaliness, and flaking were compared to baseline (day 0). The subjects

applied an amount necessary to cover the affected area with Scalp Serum daily, using a gentle massaging action. Primary endpoints of the study were efficacy of the Scalp Serum in improving symptoms of seborrheic dermatitis such as redness/erythema, itchiness, scaling/flaking, and oiliness of the scalp. Secondary endpoints included scalp soothing properties, safety, compatibility and lack of side effects. Improvements were assessed via patient questionnaires, self-examination, dermatologist examination, and standardized photography of the scalp.

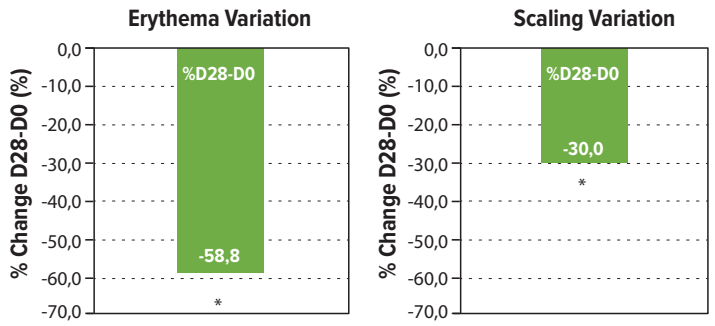

Fig. 7 Erythema and Scaling Variation.

Clinical Results Discussion

After 28 days of Scalp Serum application, there was a statistically significant 58.8% decrease in erythema scores and a statistically significant 30% decrease in scaling scores **(Figure 7)** for the Scalp Serum as compared to baseline. Although not quite statistically significant, scalp oiliness scores decreased by 16.7% compared to baseline (day 0).

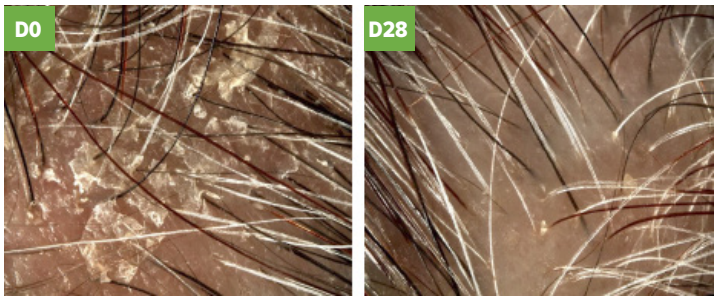

Fig. 8 Scalp soothing and relief of redness, scaling and itch with Scalp Serum (with 5% B'utyQuin)

In terms of acceptability and compatibility of the treatment, the Scalp Serum was significantly well received by subjects as it did not leave the hair oily, gave the scalp a soothing sensation, and left the scalp feeling less irritated and itchy. In evaluating scalp itchiness, redness/irritation, and scaling, at least 60% of subjects reporting significant improvement in their symptoms after 28 days of treatment, with 30% reporting complete elimination of seborrhea symptoms at the end of the study. No skin discomfort, reactions, or side effects attributed to the Scalp Serum were reported and it was felt the Scalp Serum provided a soothing effect and was rated compatible by study subjects. The Scalp Serum was therefore deemed a safe and effective aid for controlling the symptoms of SD. In summary, after 28 days of application there was a statistically significant decrease in the erythema and scaling scores for the Scalp Serum, compared with day 0 **(Figure 8)**. The product was also found to be very compatible with all subjects' skin type, with an excellent safety profile.

Conclusion

Both *in vitro* and clinical research have demonstrated the significant anti-inflammatory, antioxidant, and antimicrobial properties of a proprietary BSO with 3% thymoquinone and low free fatty acids, branded as B'utyQuin, for cosmetic and dermatologic use. In this current clinical trial, we have demonstrated the significant beneficial effects of Scalp Serum with 5% B'utyQuin, on the symptoms of mild to moderate seborrheic dermatitis in adults over a 28-day period. This blinded, controlled clinical trial demonstrated topical application of Scalp Serum resulted in significant improvements in scalp irritation, erythema, itchiness, scaling, and overall health of the scalp as compared to baseline. Reductions in scalp oiliness were also observed. These beneficial effects are likely due to the anti-inflammatory, antioxidant, and antimicrobial properties of this proprietary cold-pressed, standardized black seed oil. Modulation of mitochondrial biogenesis, ATP production, oil droplet size, and energy expenditure in fat cells are also thought to play a role in BSO's effect on symptoms of seborrhea of the scalp. This formulation containing 5% of the standardized black seed oil may have the potential to inhibit overgrowth of opportunistic pathogens to support maintenance of a healthy skin microbiome and modulate the occurrence of symptoms in those prone to seborrheic dermatitis and dandruff. It may represent a safe and effective alternative to conventional antimicrobial preparations currently in use for seborrheic dermatitis, skin and scalp discomforts and disorders.

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authors

Liki von Oppen-Bezalel, PhD
TriNutra Ltd. | Liki@trinutra.com | www.trinutra.com
Julie S. Jurenka, MT(ASCP), CCN (Cand.)

Proven Benefits on Hair growth and Nail Quality in Women of a Recently Developed Dietary Supplement

A. Derr, U. Heinrich, D. Seiler, M. R. Götz, C. Neumeister, N. Braun

abstract

Deficiency of micronutrients may represent a modifiable risk factor associated with the development, prevention, and treatment of decreased hair and nail quality or hair loss. The aim of this monocentric, single-arm intervention study was therefore to investigate whether taking a food supplement with a rational composition of components affects hair and nail quality in women. A validated and non-invasive tool, the Trichoscale® method, was used to quantify hair loss/hair growth. Subjective evaluation of hair and nail quality was also carried out by subjects and experts using a questionnaire. Intake of the dietary supplement resulted in significant improvement in hair status in several key parameters. The number of hairs in the growth phase significantly increased when compared to baseline (+7.5% after 8 weeks, +10.7% after 16 weeks), as did the density of adult fully pigmented hairs per square centimetre (+2.6% after 8 weeks; +5.9% after 16 weeks). Hair and nail quality improved significantly according to both the self-evaluation of the female participants and the assessment by a dermatological expert. The results of the Trichoscale® method and subjective evaluations consistently demonstrated the beneficial effects of a food supplement on hair growth and nail quality in women.

Introduction

The appearance of hair and nails is a major concern for women worldwide and a good head of hair is often associated with beauty and health. Hair and nails are unique structures made up of sulphur rich keratin fibres [1]. While nails are produced by the nail apparatus, each hair is produced and anchored into the skin by the hair follicle. The hair bulb, which is nourished by blood vessels, forms the base of the hair follicle in which cells divide and grow to build the new hair shaft. Hair follicles are characterised by the cyclic nature of their development. The hair follicle cycle successively goes through the anagen, catagen, telogen, and latency phases, which correspond, respectively, to hair growth, arrest, shedding, and absence before a new anagen phase is initiated [2]. Each follicle can undergo repeated cycles until it eventually dies or miniaturises.

Losing hair or a reduction in hair quality can lead to a feeling of a loss of identity and have psychologically detrimental effects, especially on women [3]. Besides hormonal changes, medical conditions, ageing or genetic disposition, and nutritional deficiency can also play a causal role. The same applies to the quality of the nails.

Vitamin and mineral supplementation is often considered as a means to prevent or manage hair loss [4]. Micronutrients such as vitamins and minerals can play an important role in normal hair follicle development and immune cell function, and their deficiency may contribute to hair loss [4]. Biotin deficiency can manifest as alopecia, brittle nails and dermatitis and low selenium leads to whitened nail beds [5]. Iron deficiency is the world's most common nutritional deficiency and has been shown to be a risk factor for hair loss in non-menopausal women [6] and koilonychia in some cases (spoon-shaped nails) [5]. Although the role of iron in hair loss is not clear, serum ferritin concentration has been shown to be lower in females with patterned hair loss [7] and treatment for hair loss has been shown to be enhanced when iron deficiency, with or without anaemia, is treated [8]. The trace elements zinc and copper play a role in hair loss and growth [9]. Zinc has been shown to be a potent inhibitor of hair follicle regression and accelerates hair follicle recovery [10]. In hair loss patients, serum zinc concentration has been shown to be significantly lower than controls [9].

Not only do vitamins and minerals have a potential effect on hair growth, but also miliacin, the main triterpenoid from millet, has been shown to have an influence. A combination of miliacin encapsulated in polar lipids was found to enhance cell proliferation in hair bulbs from the human scalp [11]. This is possibly through the stimulation of keratinocyte metabolism and proliferation [11,12]. The amino acid L-cysteine has also been focused on as a cell growth-stimulating agent [12]. Human epidermal keratinocytes derived from foreskin grown in a deficient medium without L-cysteine had a reduced metabolic capacity, whereas increasing doses of L-cysteine restored it [12].

In order to support women suffering from reduced hair and nail quality, Dr. Pfleger Arzneimittel GmbH has created an innovative vegan food supplement (BIO-H-TIN Hair Essentials)

containing vitamins and minerals which have been scientifically proven by the EFSA (European Food Safety Authority) to have an effect on the maintenance of normal hair and nails or other effects, for example, on cell division, energy metabolism or the immune system (see **Table 1**). The capsules, which should be taken once daily, contain the vitamins B7 (biotin) and B5 (pantothenic acid), the micronutrients zinc, iron, copper and selenium, the amino acid L-cysteine and millet extract. The daily reference intake values defined in Annex XIII EU 1169/2011 [13] were used as guidance for the amount of each component.

The components of BIO-H-TIN Hair Essentials are contained in a natural transparent capsule which is made of just two components, plant-based hydroxypropylmethylcellulose (HPMC) and water. The capsules are certified to be vegan, are suitable for use with organic ingredients and are non-GMO. The manufacturers of all components contained in the capsules are committed to sustainability.

Ingredient	Per daily dose (1 capsule)	% RDA [13]	Health claims / Evidence Ref. no.
Biotin	50 µg	100	[14]
Zinc	5 mg	50	[15]
Iron	7 mg	50	[16,17,18]
Copper	1 mg	100	[19]
Selenium	55 µg	100	[20]
Pantothenic acid (Vitamin B5)	6 mg	100	[21]
L-cysteine	4 mg	--	[12]
Millet extract	17% of the total product	--	[11,12]

Table 1: Vitamins and nutrients contained in BIO-H-TIN Hair Essentials [13]

The choice of ingredients was carefully considered not only regarding aspects such as nutritional properties or authorised health claims, but also on the quality of the ingredients with respect to origin, bioavailability, sustainability and tolerability. As a source of iron, ferrous bisglycinate is contained in the product. Bisglycinates are salts of the endogenous amino acid glycine. Here, the central element is surrounded by 2 organic glycine molecules and chemically the fully reacted complex constitutes a chelate. The amino acid chelate is digested only in the intestine, which optimises the absorption. Ferrous bisglycinate is absorbed intact into the mucosal cells of the intestine where it is hydrolysed into its iron and glycine components [22,23]. Mineral bisglycinates are highly stable chelates developed to provide a safe product with improved bioavailability. Their use also decreases the occurrence of gastro-intestinal side effects such as epigastric pain, nausea, vomiting and diarrhoea [22]. The zinc and iron dosages were carefully adjusted to be below the reference daily allowance (RDA) to avoid impaired absorption and at the same decrease the theoretical risk of over-supplementation. The L-cysteine used in the product is plant-based from sustainable raw materials and was produced in a sustainable fermentation process, for which the patented technique was awarded an environmental prize. The millet extract, which accounts for 17% of the product, is from an organic source and does not contain any GMO.

Methods

The study was conducted as a monocentric, single-arm, non-placebo-controlled intervention study of a dietary supplement. The aim was to investigate the efficacy and tolerability of the

product. The study design was approved by the Ethics Committee of Witten/Herdecke University before the study began. Included in the study were 42 healthy female subjects aged 30 to 50 years (mean 44.8 years) who suffered from mild hair loss or desired an improvement in hair and nail quality. The prerequisite was that they fulfilled all inclusion and exclusion criteria and maintained their usual dietary habits.

Study conduct

Following an initial examination, the study participants received the study preparation BIO-H-TIN Hair Essentials for a period of 16 weeks, which was to be taken once a day with a main meal. In the course of the study, measurements and subjective assessments were collected after 8 weeks and 16 weeks.
The hair status was measured using the Trichoscale® method [24]. The measurements were supplemented using a questionnaire with a grading scale by self-evaluations of the subjects on hair and nail quality as well as an expert rating.

Determination of hair status with the Trichoscale® method

The growth cycle of hair consists of three phases: an active growth phase (anagen phase), a transition phase (catagen phase) and a resting phase (telogen phase). The Trichoscale® method used here (Fotofinder Systems, Germany) is a validated and non-invasive instrument for quantifying hair loss/hair growth/hair density and represents a further development of Hoffmann's TrichoScan® method [24].

The Trichoscale® software is based on the assumption that the anagen hairs grow about 0.3 mm/day, while the telogen hairs show no growth. By means of a corresponding software, the number of hairs per test field, the hair density [hair /cm²], the anagen rate [%], the telogen rate [%], as well as the number of terminal hairs are calculated automatically.

The TrichoScale® method allows diffuse hair loss to be digitally documented and quantified. From these data, for example, an improvement of the hair status (increase in the anagen phase, i.e. the growth phase of the hair) can be determined.

Expert Evaluation

A defined expert evaluation took place. The quality of the hair and nails was assessed by the expert at the beginning of the study (T0), after 8 weeks (T8wk) and after 16 weeks (T16wk) of taking the food supplement under investigation.

Self-assessment of the volunteers

In addition, the subjects were asked to complete a self-assessment questionnaire at baseline (T0), after 8 weeks and after 16 weeks of taking the investigational food supplement. They were asked to rate their hair and nail quality and to make an assessment of hair loss using a grading system.

Results

In the following, the results of the study course are shown in relation to the hair status with anagen rate (%), the telogen rate (%) as well as the number of terminal hairs. In addition, the results of the expert and self-assessment are listed in **Table 2**.

Anagen rate [%]

The anagen rate (hair in the growth phase) in the test field increased in the course of the study from an average of T0 = 71.6% to 77.0% (T8wk) and to 79.3% (T16wk) **(Figure 1a)**. This corresponds to an increase in the anagen rate in the test field of 7.5% and 10.7% respectively **(Figure 1b)**. The results are statistically significant.

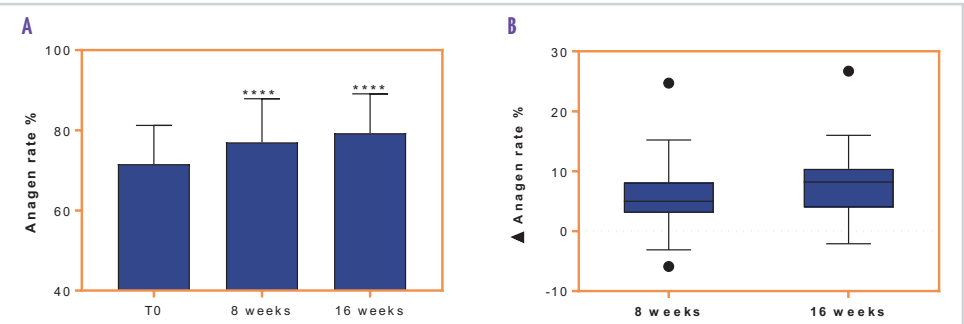

Fig. 1 **Anagen rate [%]** (**** designate statistical significance)

Telogen rate [%]

The telogen rate (hair in the resting phase) in the test field reduced in the course of the study from an average of T0 = 28.4% to 23.0% (T8wk) and 20.7% (T16wk) **(Figure 2a)**. This corresponds to a decrease in telogen rate in the test field

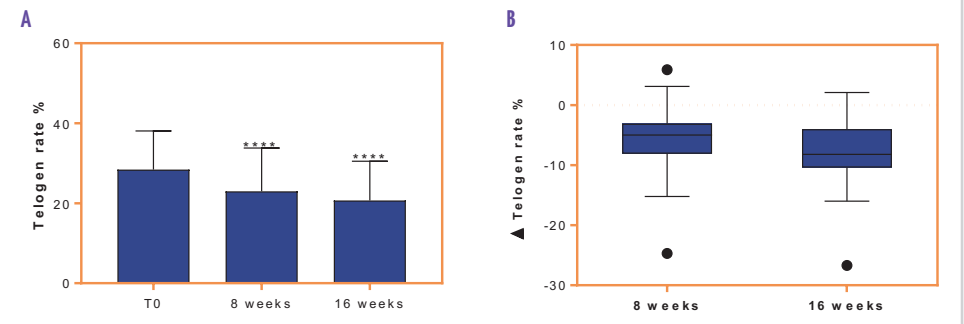

Fig. 2 **Telogen rate [%]** (**** designate statistical significance)

of 19.1% and 27.1% respectively **(Figure 2b)**. The results are statistically significant.

Number of terminal hairs / test field

The number of terminal hairs in the test field increased over the course of the study from an average T0 = 101.4 to 103.9 (T8wk) and to 107.4 (T16wk). This corresponds to an increase in terminal hairs in the test field of 2.5% and 5.9% respectively. The results were statistically significant after 16 weeks.

Expert evaluation of effectiveness

Compared to the baseline examination, all parameters of the dermatological evaluation for the examination after 16 weeks decreased on average. A decrease in the arithmetic mean corresponds to an improvement in the corresponding assessment. The changes were statistically significant for most of them (highlighted in lilac). This trend can already be seen for most parameters after 8 weeks. The results of the expert evaluation are shown in **Table 2**.

Parameter	Difference	Expert evaluation		Subjects' self assessment	
		Arithm. mean	p-value	Arithm. mean	p-value
Hair loss	T8wk - T0	-0.610	<0.0001	-0.585	0.0004
	T16wk - T0	-0.897	<0.0001	-0.976	<0.0001
Hair appearance	T8wk - T0	0.049	0.7744	-0.366	0.0044
	T16wk - T0	-0.436	0.0036	-0.707	<0.0001
Hair thickness	T8wk - T0	-0.220	0.0117	-0.146	0.3205
	T16wk - T0	-0.359	0.0002	-0.585	<0.0001
Haar strength	T8wk - T0	-0.537	0.0026	-0.463	0.0013
	T16wk - T0	-0.205	0.0963	-0.805	<0.0001
Hair shine	T8wk - T0	0.732	<0.0001	-0.341	0.0187
	T16wk - T0	-0.154	0.2657	-0.561	<0.0001
Hair combability	T8wk - T0	0.171	0.2207	-0.317	0.0204
	T16wk - T0	-0.308	0.0332	-0.707	<0.0001
Hair quality	T8wk - T0	-0.707	<0.0001	-0.366	0.0018
	T16wk - T0	-0.513	<0.0001	-0.707	<0.0001
Hair damage	T8wk - T0	-0.171	0.1295	-0.463	0.0011
	T16wk - T0	-0.205	0.1344	-0.561	<0.0001
Hair volume	T8wk - T0	-0.415	0.0003	-0.220	0.0979
	T16wk - T0	-0.359	0.0005	-0.805	<0.0001
Hair growth	T8wk - T0	-0.317	0.0526	-0.366	0.0238
	T16wk - T0	-0.641	<0.0001	-0.780	<0.0001
Nail appearance	T8wk - T0	-0.390	<0.0001	-0.439	0.0029
	T16wk - T0	-0.846	<0.0001	-0.780	<0.0001
Nail strength	T8wk - T0	-0.537	<0.0001	-0.683	<0.0001
	T16wk - T0	-0.974	<0.0001	-1.049	<0.0001
Nail quality	T8wk - T0	-0.415	<0.0001	-0.537	<0.0001
	T16wk - T0	-0.846	<0.0001	-0.829	<0.0001
Nail growth	T8wk - T0	-0.073	0.5875	-0.317	0.0310
	T16wk - T0	-0.667	<0.0001	-0.780	<0.0001

Table 2 Results of expert evaluation and subjects' self-assessment of hair and nails. Statistically significant differences are highlighted in lilac.

Self-assessment of the effectiveness by the subjects

Compared to the baseline examination, all parameters of the self-assessment by the subjects for examination decreased after 16 weeks. The changes were statistically significant for almost all of them (highlighted in lilac). This trend can already be seen for most parameters after 8 weeks.

Overall, it has been shown that for all parameters of the subjective evaluation by the subjects, that this effect is even more pronounced than for the dermatological evaluation by the expert. After 16 weeks, all evaluations of the subjects were positive/significant. The results of the subjects' self-assessment are shown in **Table 2**. As can be seen in **Table 2**, the expert and subjects' self-assessment were nearly always identical in their significance.

Summary of results

The intervention study with the product under investigation showed high efficacy in improving hair status, with significant improvement in anagen and telogen rates as well as terminal hair. In addition, the expert rating and the self-assessment of the participating subjects showed a positive trend in the evaluation of hair and nail quality with statistical significance. Furthermore, a good tolerability could be determined, none of the 42 subjects showed intolerances during the course of the study.

Discussion

The number of food supplements on the market is immense, as is the range of ingredients. Food supplements, which are regulated as foods in the European Union (EU), are carefully controlled by the EU which lays down a list of vitamins and minerals that may be added for nutritional purposes in food supplements [25]. The European Food Safety Authority (EFSA) has provided authorised health claims, i.e. an evidence based statement about a relationship between food and health, for certain foods which may also be used for food supplements. The present study was carried out to prove that intake of a food supplement with ingredients which were carefully chosen because of their known positive effects on hair and nail growth did actually have a beneficial effect. The study not only used a subjective evaluation by both experts and subjects, but also a validated technique, the Trichoscale® method, to assess hair growth. The results of the subjective evaluations that hair volume and growth were improved after 4 months of daily use of BIO-H-TIN Hair Essentials were confirmed by the Trichoscale® method which decisively showed that the number of hairs in the anagen phase and the anagen rate increased significantly during the study, as did the number of terminal hairs. As the telogen rate was reduced the total number of hairs was also increased as they were retained for longer in the scalp. No participants stopped taking the product due to adverse events proving its excellent tolerability.

In this study the Trichoscale® method was used for measurement of hair growth and hair cycle. The advantage of this method is that it can be performed non-invasively, however, experience in implementation is important and the software must be corrected manually for hairs that lie on top of each other [26]. The subjective assessment of hair and nail quality used in this study also relies on human judgement and there is often great variability in the outcome. One further critical point of the study is that it was not placebo controlled. This might be compensated by the fact that the test subjects were living on a normal diet before, thus had a nutritional status which can be considered typical for the general (female) population. Nevertheless, subjects still complained about their deficient hair and nail status. The statistically significant results after the 16 week treatment period prove a definite positive beneficial effect on hair and nail quality when BIO-H-TIN Hair Essentials is taken once daily.

In general, there is limited information available on the effects of complex supplementation with nutrients such as iron, zinc or selenium in the absence of deficiency [27]. Although biotin deficiency has been found in up to 38% of women complaining of hair loss [28] there are no evidence based data that supplementing biotin promotes hair growth [4]. Iron deficiency is common in females and while most studies support supplementing iron in patients with low ferritin levels, there is no consensus on "normal ferritin" levels [4]. It has also been suggested that supplements may actually prove harmful to hair in the absence of deficiency and that toxicity can occur with excess supplementation [27]. Although there are conflicting views regarding minerals and hair loss, this study has proved that hair growth and volume were significantly increased following intake of the food supplement. The effectiveness of the individual ingredients or concentrations can however not be assessed, or whether it was the interaction and concentration of the mixture of micronutrients and vitamins which led to the beneficial effects. Superiority to other food supplements can also not be speculated on as no results are available. The EFSA has not provided an evaluation or positive health claims on the benefits of any combinations of vitamins and minerals. The results here demonstrate that claims for combinations of micronutrients could be considered.

The components contained in BIO-H-TIN Hair Essentials are also taken in during a normal diet, but whether or not in sufficient quantity is questionable. As the body cannot produce some of the trace elements itself such as copper, the intake of copper through food, food supplements or medicines is essential. Simply using the 100% RDA of certain vitamins and micronutrients is however not always beneficial and iron supplementation has been shown to cause gastrointestinal complaints which can be avoided if only 50% RDA is used. As no adverse events were reported during the study, the concentration of the components in BIO-H-TIN Hair Essentials appears to be very well tolerated.

Interactions of micronutrients can affect absorption and bioavailability of other nutrients by a number of mechanisms [29]. The consequences of these interactions may depend on the relative concentrations of the nutrients. In competition studies, it has been shown that copper and zinc inhibited iron uptake, and while iron inhibited copper uptake, zinc did not. When the three metals were given together (1:1:1 ratio), iron or copper uptake was inhibited approximately 40%. These results point to a potential risk in the absorption and bioavailability of these minerals by the presence of other minerals in the diet and is an aspect which must be considered in food supplementation [29]. Oral iron therapy has been shown by other authors to impair zinc absorption in a dose-independent fashion but does not affect copper absorption [30]. For this reason, the minerals iron and zinc were not used at 100% RDA.

Conclusions

In conclusion, taking a dietary supplement containing a balanced mix of vitamins and micronutrients can improve hair and nail quality in females.

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contact

Dr. Pfleger Arzneimittel GmbH
Dr.-Robert-Pfleger-Str. 12 | 96052 Bamberg | Germany
www.dr-pfleger.de

Aerobic Spore-Formers (*Bacillus spp.*) as Contaminants of Cosmetic Products

U. Eigener, J. Nussbaum

abstract

Aerobic spore-formers of the genus *Bacillus* are found as contaminants of cosmetic products. Therefore, the primary target is to avoid contaminations with these microorganisms. For this purpose, requirements are needed for raw materials-quality as well as for the hygiene-system in the production areas. Additionally, growth of these contaminants has to be avoided through an effective preservation of product-phases and the finished product. Usual preservative systems can be assumed to be effective against these spore-formers. Cosmetic products containing *Bacillus*-counts in conformity with the limits given in ISO 17516 do not present a risk for the product or the health of the user. This applies for products used under usual conditions, and if the microorganisms are not able to multiply in the product and if the conformity of the batch with the required microbiological limits is proven in adequate control tests.

Introduction

Cosmetic products must be in conformity with defined microbiological quality requirements to guarantee the product`s characteristics and to avoid health risks for the user (Cosmetics Regulation) [1]. Basic demands in this context are the ones given in the norm ISO 17516 [2], which defines limits as TVCs (total viable count; aerobic microorganisms) and for the absence of specified microorganisms. For the latter group a number of bacteria and fungi, which could cause a health hazard, are mentioned by way of example as "indicator microorganisms", but practice microorganisms presenting a risk for the product integrity and/or the health of the user should be added. All others are included in the TVC. This group often also contains aerobic spore-formers of the genus *Bacillus*. They are found in varying numbers and may therefore also be relevant for the product approval. It is therefore advisable to take a closer look at this group of microorganisms. This makes it possible to understand how these microorganisms contaminate the product, to take effective product protection measures and to make a valid risk assessment.

1. Characteristics of the *Bacillus* species

Bacteria of the genus *Bacillus* (family: *Bacillaceae*) are gram-positive rods, some of them are peritrichously flagellated. Most of them live under aerobic conditions, some of them are facultative anaerobes, and are found ubiquitously. The genus is able to build endospores. Most species of aerobic spore-formers belong to the genus *Bacillus*, but others are found in further genera of the family *Bacillaceae* or other families of the order *Bacillales* (e.g. *Paenibacillaceae*) [3]. The endospores are not a form of multiplication, but they are just highly resistant cells formed to persist unfavourable environmental conditions. Multiplication is bound to the status of the vegetative cell. Spore-forming and germination are very complex processes. Each cell can form one endospore, which is characterized by a dehydrated status, different chemical structures and enzymatic provisions and specific physiological functions. Additionally, the spore builds a thick wall consisting of different layers. All this makes the spores very resistant and enables them to remain viable in a dry state over long time periods of unfavourable conditions (nutritional and water deficiency, high temperatures, UV-radiation). In case of appropriate physiological conditions, the spore can give rise to a germination process which will lead to a vegetative cell again. Also, short heating can initiate the germination.

Compared to vegetative cells, the spores are very resistant against antimicrobial substances and high temperatures, and therefore sterilization can only be achieved with temperatures ≥121°C or ionising radiation, and just selected disinfection-agents (e.g. peracids, glutardialdehyde, chlorine) are able to kill endospores.

Only very few species of the genus *Bacillus* are known as hazards for human health. This is especially *B. anthacis*, the anthrax agent. *B. cereus* appears as contaminant of food, but only high numbers ($>10^4 - 10^5$ cfu/g) cause health risks through the production of toxins (gastrointestinal disease) [4]. *B. cereus*, however, is also mentioned in connection with other infections (e.g. eye-infections, post-operative wound infections, other opportunistic infections) [5, 6, 7, 8]. Accordingly, *Bacillus* species are assigned with very few exceptions (e.g. *B. cereus*-group) to the risk-group 1 in the TRBA 466 (2015) [9].

Spore-formers of the family *Bacillaceae* are primarily of importance as spoilage bacteria in the food area. Specific growth conditions (e.g. temperatures, oxygen demand, specific nutrients) and differing metabolic reactions are used to classify the various species [10]. Other relevant species, for instance, are *B. licheniformis*, *B. subtillis*, *B. coagulans* and spore-formers of further genera like *Geobacillus*, or genera of other families like *Paenibacillus* or *Alicyclobacillus*. In high numbers, aerobic spore formers as contaminants in cosmetics can lead to the destruction of the product, resulting in odour problems and / or the structural degradation of the product.

2. Appearance in cosmetic products

Aerobic spore-forming bacteria are regularly found when cosmetic products of various types are examined for the microbial content. A systematic overview of *Bacillus* species can, however, not be given since the targets, the methods and the identification of microorganisms in these studies vary a lot. Our own experience over many years confirms that such spore-formers are common. Recent data published by the BAV Institute [11] show a high percentage of gram-positive bacteria in tested product-samples showing a microbial contamination, and many of them are aerobic spore-formers.

Such results can be explained when looking at contamination sources and routes of this group of microorganisms. *Bacillaceae* are found mainly in soil and as such in materials of natural kind and then in dirt and dust. Consequently, endospores are often present in dry environments and are commonly distributed via air. Therefore, they are also found as usual contaminants in production areas.

Bacillus species frequently occur as contaminants of cosmetic raw materials. The appearance of these contaminants depends a lot upon the origin, but also the kind and production- conditions of the raw materials. Raw materials of natural plant or mineral origin are often highly contaminated with microorganisms including aerobic spore-formers. Powder raw materials which have undergone a drying process – especially drying with high air stream – frequently contain endospores because they are able to sustain increased temperatures and drying effects. The microbial count in raw materials can effectively be reduced through sterilisation processes (e.g. ionizing radiation).

2.1 Detection and identification of aerobic spore-formers

Culture media usually employed for the microbiological examinations of cosmetic products are also suitable to detect *Bacillus*-species (e.g. CASO-agar or CASO-bouillon, incuba-

tion temperature 30°C). For the identification Gram-staining, catalase reaction and the formation of endospores (microscopy) should be used [10]. Further biochemical reactions can be employed to differentiate various species. It is recommendable to include the genus *Bacillus* into the tests for the validation of microbiological detection methods. *B. subtilis* can be used for this purpose as suggested for tests with non-sterile drugs [12].

Flagellated *Bacillus*-species may swarm on culture plates resulting in an "overgrown surface". This could even be the case if the numbers are low but the impression of high numbers is given. This should be taken in mind when *Bacillus*-species are present, and an intermediate reading could be helpful if applicable, to avoid false results.

3. Behavior of the contaminants in the product

Bacillus-species as contaminants in cosmetic products can be present as vegetative cells or as endospores. A spore germination may take place if appropriate physiological conditions are present. Relevant factors in this context are the availability of water and suitable nutrients, which usually are sufficiently provided with the cosmetic ingredients. The vegetative cell develops out of the spore and can then lead to multiplication. This development can be inhibited by means of antimicrobial measures (extreme pH-values, toxic redox-value, antimicrobial substances), because the cells do not have the high resistibility as found with the endospores.

3.1 Protection measures in the production area

First of all, a product contamination through spore-forming bacteria has to be avoided during the production process. In order to achieve this target, special attention has to be paid to the microbial content of raw materials. Therefore, appropriate microbiological specifications have to be agreed upon with the raw material supplier, and respective control tests have to be performed upon raw material delivery. Use and storage conditions for the raw materials have to be defined in such way that no additional contamination problem can occur. Further adequate hygiene measures have to be taken in the following production steps. Such measures are for instance: careful cleaning of rooms, appropriate air filtration systems and a suitable materials flow with respective air locks. With respect to contamination problems through spore-forming bacteria, special attention should be paid to the distribution via air and through dust sedimentation – for instance when filling tins and jars. Furthermore, regular monitoring activities should be installed that can provide information about possible contamination problems in the relevant workshop areas.

An important aspect is to avoid multiplication of contaminants in all steps of the manufacturing process. Harmlessly low numbers of spore-formers in raw materials (e.g. powder materials) may become a substantial problem if they are allowed to grow and increase in numbers. This is possible if aqueous dilutions of the raw materials are prepared or the raw materials are part of unpreserved product phases and subsequently these dilutions/phases are stored for too long before being used in the next production step.

3.2 Protection through preservation

As mentioned before, it is important to ensure that microorganisms in the product are not able to multiply. This is usually achieved by means of effective preservation. Aerobic spore-formers, however, do not belong to the standard test-spectrum of microorganisms when applying the PET (preservative efficacy test) according to ISO 11930 [13]. This results from the long-standing experience that *Bacillus* species have not been identified as substantial risks in cosmetics and do not show an exceptional resistance against the usual preservatives [14,15]. Due to missing data about PET-results, very little information can be found about the efficacy of preservatives/preservation systems against the *Bacillus* species. The available results with antimicrobial substances resp. groups of substances, however, affirm the above-mentioned evaluation. Examples are given in the following studies: benzoate/sorbate [16], parabens [17], essential oils [18], ZnO [19]. Also, an FDA-study with "non-classically preserved" near-eye products assessed a contamination with aerobic spore-formers (only given as genus *Bacillus*) not as a critical hazard – a small percentage of the products was found with slightly increased numbers (> 100 and ≤ 500/g) [8].

Our own PET results indicate that in the case of adequate preservation acc. to ISO 11930, a good efficacy against cells of aerobic spore-formers is obtained as well. An example of such test-results is given in **Figure 1 A/B**. Besides the bacterial standard test-microorganisms, vegetative cells of two *Bacillus* species have been used in these tests (*B. subtilis* (ATCC 6633)

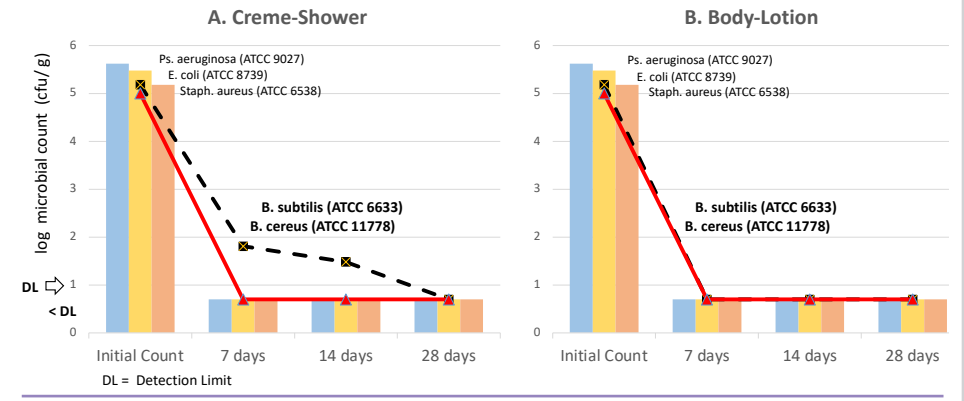

Fig. 1 PET - Results

and *B. cereus* (ATCC 11778)). The spore-formers have been pre-cultivated (5.2.2.4.2 Culture medium for bacteria) and used in the tests as given in ISO 11930 [13]. Two samples from the trade were employed as test products: Creme Shower (preservation: organic acids and benzyl alcohol) and Body Lotion (preservation: organic acids). In both cases an adequate and comparable preservation efficacy is obtained with the *Bacillus* species as found with the other gram-positive and gram-negative test-bacteria.

4. Safety assessment in case of *Bacillus*-contaminations

Aerobic spore-formers as contaminants of cosmetic products are well known for a long time period, but such contaminations have usually not been classified and described as serious hazards. Accordingly, the *Bacillus* species are not included into the group of "specified indicator microorganisms", and therefore the general limit value as given in the ISO 17516 (2) should be applied. The producer must of course to make sure that the microbial counts can be reliably maintained and no growth takes place. Consequently, an effective preservation (ISO 11930 [13]) resp. a microbiological stability acc. to ISO 29621 [20] and adequate controls (for conformity with instructed procedures and for the microbiological count, in process-controls and finished product-controls) are of basic importance. Adequate instructions and the performance of such measures should also be verified as part of the safety assessment.

Because the genus *Bacillus* is not generally part of the PET, the question has to be asked how far these test results include the evidence of the preservation efficacy against these bacteria. Our own experience and literature data (see also chapter 3.2) do not show a higher resistance of *Bacillus*-cells to the commonly used preservation systems compared to the standard test microorganisms. Therefore, the conclusion that standard PET results also cover efficacy against *Bacillus* cells can be considered justified.

With regard to aerobic spore-formers, a special situation may result from the fact that extremely resistant endospores may be found as contaminants of the product. Even though these spores cannot multiply, a germination and a resulting multiplication may take place in insufficiently protected raw material dilutions and product phases or in the cosmetic product which has not been effectively preserved. Accordingly, in addition to the prevention of product contamination (through keeping to hygiene-rules and ensuring good raw material-quality), adequate process procedures must be defined, an effective preservation and appropriate controls (raw materials, productions steps and finished product) must be employed to avoid microbiological risks. Testing for microbial counts must also be applied for products which are classified as "low risk products" (acc. to ISO 29621), since endospores may also survive under these conditions. This aspect is especially relevant for products containing critical raw materials (see above).

Since there is no general requirement for cosmetic products to be sterile, a risk assessment must be performed regarding the potential hazard caused by microbial contaminants. First of all, attention should be paid to the fact that in any case only low to medium counts are allowed according to ISO 17516. For instance, spoilage of the product will occur only in case of high numbers of Bacillus-species ($> 10^4 – 10^5$ cfu/g). The health risk due to the use of cosmetic products contaminated with acceptable numbers of aerobic spore-formers can be neglected as extremely low [7]. In the risk assessment, it should be noted

that aerobic spore formers have seldom been found in connection with infections. Also, the type of product, its application, the user group and underlying health conditions should be taken into account [6,21]. Since cosmetic products are not sterile, their use should generally be limited and performed with special care in areas where specific medical requirements are needed (treatment under sterile conditions, contacts and treatment of persons in vulnerable status). For the common user of cosmetic products and under normal conditions of use, a serious health risk cannot be assumed in the event of contamination with aerobic spore-formers [6,7]. The FDA-study [8], for instance, classifies products for the use around the eyes, which were contaminated with low to medium numbers (≤ 500 cfu/gr) of *Bacillus* species (different species or without identified species), not as risk from a health point of view.

According to today's knowledge, cosmetic products contaminated with aerobic spore-formers do not present a general risk. This is true as long as the microorganisms are present in low numbers (not exceeding the limits given in ISO 17516) shown in adequate product controls and as an effective preservation prevents multiplication in the product.

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Authors

Dr. Ulrich Eigener | Hamburg | Germany
ulrich.eigener@t-online.de

Joelle Nussbaum | BAV Institut GmbH | Offenburg | Germany
joelle.nussbaum@bav-institut.de

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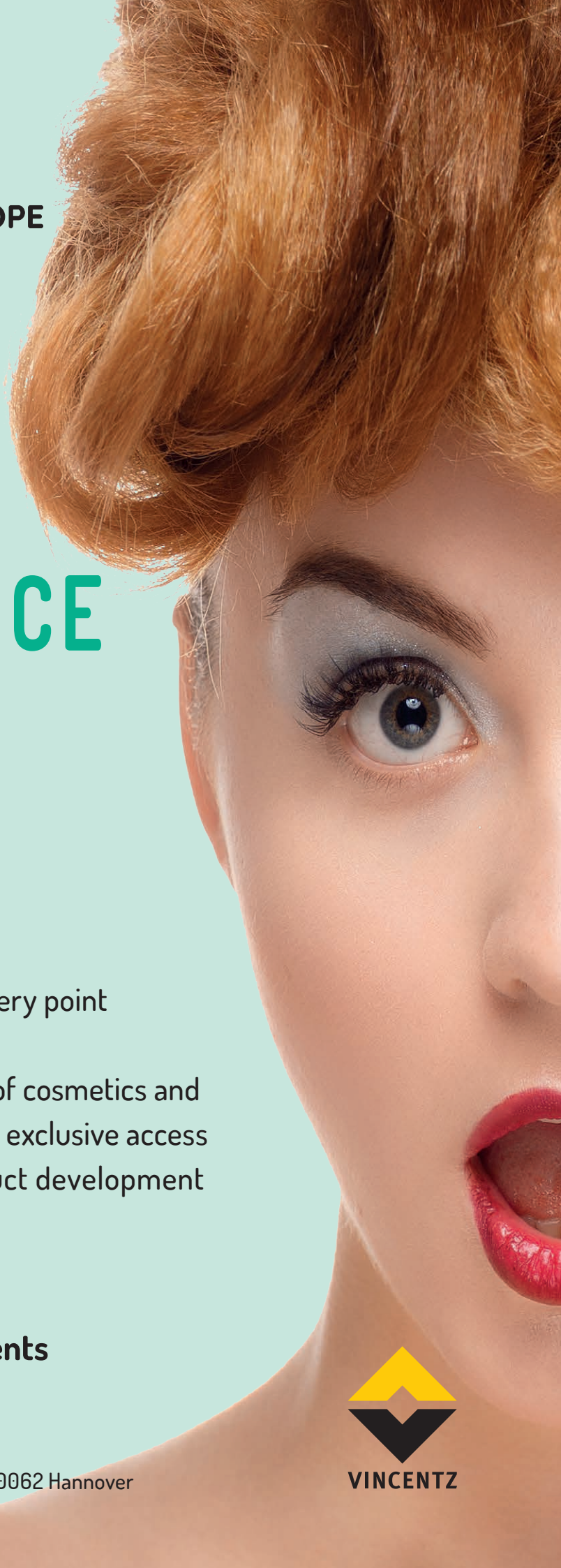

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How In-line, Real-time Rheology Sensors Can Provide Agile Manufacturing in the Personal Care Sector

T. Machin, R. Travis

Rheology is the study of the way a material flows and is a key component in the manufacturing process of any liquid product. It is vital that this property is measured in the manufacture of household and personal care ingredients to enhance operational efficiency and ensure final product quality. Without the correct rheology, items like washing up liquid and shampoo would look and feel very different.

As manufacturers in the personal care industry look to improve process efficiency and grow with demand, a new era of measurement technology is emerging. Real-time, in-line rheology measurements are supporting manufacturers with their efforts to innovate, create new ways to get their products to market, release capacity, and improve quality and agility in their manufacturing base.

With the growing trends of e-commerce and a dynamic consumer market, **Rob Travis** and **Thomas Machin**, of Stream Sensing, discuss the importance of real-time process measurements in agile manufacturing. They take a look at how electrical resistance sensing can be used to monitor rheological properties, within the process line and look at the benefits which this emerging digital process technology could have across the sector.

Explosion in E-Commerce

While consumer habits in the personal care and household sector had already begun to shift towards online sales, prior to March 2020, the COVID-19 pandemic accelerated this with many personal and homecare manufacturers embracing an e-commerce approach. Increasingly, consumers gain their information from influencers and communities when choosing products, which has led to an increase in online purchasing. Consumers also have greater awareness of, and concern about, the ingredients that these products contain, meaning that manufacturers have begun moving towards direct-to-consumer (DTC) marketing models. As a direct result, manufacturing facilities must become more agile to meet the ever-changing demand.

Online share of sales in the personal care industry continue to grow, and is projected to jump to 48% in the United States by 2023 [1]. A 2021 survey of 2,000 consumers of health and beauty products, found that 67% and 80% of beauty and health and personal care consumers, respectively, had turned to online purchasing [2]. This rapid expansion through digital channels is fueling growth in the beauty industry, and, according to a recent report, e-commerce sales of personal care products are predicted to grow to 12% compound annual growth rate (CAGR) from 2021 to 2026. This is proceeding to outpace growth in bricks-and-mortar sales [3].

From DTC to Product Personalisation

Aligned with the growth in e-commerce platforms, manufacturers are moving towards creating relationships and connecting directly with consumers. With up to 57% of manufacturers having adopted this approach, DTC is the fastest-growing e-commerce category [4].

Bypassing physical retailers and selling DTC allows manufacturers to collect consumer data including customer preferences, skin type, allergies and geographies. This information enables brands to leverage data to their advantage – meeting their customers' ever-changing needs, by adapting process formulations or production schedules. This approach requires the manufacturing process to be agile, with measurement technologies becoming increasingly important. If production has to change rapidly, according to customer demands, in-line rheology measurements become critical to ensure that the final product quality remains within specification.

This consumer data (gathered, for example, via quizzes around factors such as lifestyle, and various aspects of hair texture) can be used to provide more tailored products, which is another rising trend in the cosmetics and personal care industry.

Companies that do not adopt the DTC business model to keep pace with customer preferences, risk shrinking sales and missed opportunities for growth and expansion. A 2019 study of the top 100 cosmetic brands found that while traditional brands experienced a 7% decline in sales between 2017 and 2019, DTC brands realised an average 11% year-over-year growth [5].

Manufacturers that fail to strengthen DTC relationships in this way may not just miss out on customer retention, but also customer lifetime value (LTV). In a 2017 survey of 1,000 consumers aged 18 to 64, 80% of respondents said they were more likely to engage with a company if it offered personalised experiences [6]. Furthermore, according to an analysis of billions of transactions within a cooperative of 2,700 retail organizations, consumers who found personalised experiences appealing were ten times more likely to make more than 15 transactions in one year [6].

Data-Driven Product Development and Innovation

Using consumer data is an enabler for a brand to adapt production schedules to manufacture tailored products – a specific shampoo or conditioner, for example – customised to the traits of a consumer group's hair type, depending upon demand.

This type of personalised data gives insight about consumers who have previously been overlooked or neglected – for example, women with Afro-Caribbean hair – and then tailor short-run batch formulations to their needs. Brands can also use customer data to upstream in research and development (R&D) efforts to drive new product development or improve existing products or formulas. For instance, if consumer feedback revealed that a certain proportion of consumers of a facial cream had concerns about fine lines, a manufacturer could respond by creating a new product that targets these issues. The ability to pivot and fill such niches, however, hinges on product analysis and in-line process monitoring.

Digital processing, one of the hallmarks of Industry 4.0, plays an essential role in enabling brands to successfully adapt to a DTC model, and continuously hone the degree of customisation they're able to offer to consumers. Manufacturers must have the latest technology to collect and analyse both consumer and manufacturing data, solicit feedback, measure demand, identify markets with growth potential and then match with production capability in plants. Digital processes directly contribute to increased production, and productivity, according to Deloitte [7]. Most manufacturers are waking up to and acting on the fact that digital transformation is no longer optional, but essential for brands to remain competitive. According to a 2021 report, 88% of manufacturers anticipated the use of

smart devices and embedded intelligence in their production processes would increase within two years [8].

Ecommerce allows manufacturers to gather information on an individual customer's needs, which in turn can be fed into an algorithm that provides the consumer with a custom product recommendation based on their responses. According to the June 2019 Atlantic article, 'The Future of Marketing is Bespoke Everything', personalised beauty brands often allow customers to opt-out of fragrances, or ingredients derived from animals. People with allergies and skin sensitivities are another group that can benefit from product recommendations tailored to their needs. Increasingly, consumers are also concerned about the sustainability and safety of ingredients in cosmetics and personal care products, and are willing to switch allegiances to brands that respond by customising their products to suit their preferences. For example, according to a 2020 report, one beauty manufacturer saw sales of one of its facial creams increase by $5 million after changing the formulation to remove one of these compounds [9].

An in-line rheology sensor is one example of a digital Internet of Things (IoT) technology that can help manufacturers react to changes in the process line.

Thomas Machin
is the Senior Technology Officer at Stream Sensing. He studied chemical engineering at the University of Birmingham, where he went on to gain a doctorate in 2020 and develop the technology that Stream Sensing is now helping the industry to use. Tom has recently received charter member status with the Institute of Chemical Engineers (IChemE).

Rob Travis
is Business Development Director at Stream Sensing, with a primary responsibility of leading the sales efforts for the company, having joined in February 2022. Prior to joining Stream Sensing, Rob held global account roles with Videojet Technologies, part of Danaher Corp., and Schneider Electric.

Limitations of Traditional Rheology Measurement

Rheology is the study of the flow of matter – primarily liquids – under the influence of force or stress. (Viscosity is one of the qualities that rheometry sensors help to measure). It's also critical to the success of liquids in beauty, personal care, and household products, such as washing up liquid and shampoo. If rheology is not measured properly, a product might not have a consistent texture from one batch to the next, or it might be unsuitable for its intended purpose. For instance, if lipstick is too thick, it might cake rather than spread smoothly. Consequently, rheology can affect everything from customer satisfaction to waste due to the rejection of poor-quality products.

Manufacturing companies undoubtedly recognise the importance of rheology, yet many may not be able to fully benefit from its potential because conventional methods for measuring it are time-consuming, labour-intensive, and of variable accuracy. Off-line rheology samples are taken from the process and then analysed in a quality control laboratory. This takes time – adding around 30 minutes per batch prior to release – and skilled manpower. Taking samples can also lead to product contamination, and because the sample is taken at a single point in the process, it may not be truly representative of the final batch. There is also experimental uncertainty in running and maintaining the complex analytical equipment. Not only this, if the analysis shows the rheology of the product does not meet specifications, it will often need to be discarded or reworked, using up additional resources, such as energy, and potentially generating waste. Then there's the fact that these measurements must be made multiple times. This can add up to large volumes of waste: in fact, we have estimated that the annual product loss in personal care manufacturing globally is equivalent to 2 million bathtubs full, or approximately 320 million litres.

Reimagining Personalisation with In-Line Rheology Sensors

However, a new process technology that incorporates in-line rheometry sensors and artificial intelligence enables automated, in-line rheology measurement for fluid process optimisation. The sensors, which are built on a technology known as Electrical Resistance Rheometry (ERR), work in a fashion similar to that of a computed tomography (CT) scan. Yet, the ERR sensors use electrical fields rather than x-rays, to 'see' inside pipes and vessels, and obtain real-time rheological information about process fluids.

The addition of artificial intelligence algorithms also makes it possible to capture complex fluid behaviours often observed in industrial fluids, such as shear-banding. The instrument can be used to provide real-time data to the plant's control system for control and optimization through digital communication protocols, such as Ethernet-IP, and a user interface allows operators to measure rheology at the press of a button.

With the ability to monitor rheology in-line, and in real-time, rheometry goes from being a quality check at the end of the process to being a fully automated and controlled process. Manufacturers can easily ensure each batch is made to spec-

ifications, or a continuous process is within the correct specifications, and improve efficiency from production through to packaging. The sensor can show when a new formulation is ready to pack, meaning manufacturers do not need to extract the liquid and test it away from the process line.

Altogether, these properties will make it far more feasible, practically and economically, for manufacturers to personalise their products; for example, by monitoring changes in formulation or ingredients in real-time, tailored to customer preference. It will particularly impact short run products, enabling more efficient manufacturing of niche items. Alongside this, the generated data may foster increased innovation for R&D and product formulation through a deeper understanding of manufacturing processes, and offer greater versatility through the use of sophisticated processing equipment. To add to this, in-line rheology measurement should sharply reduce the amount of product discarded due to quality issues, and, therefore, waste, and cost.

Flowing Towards Greater Agility and Adaptability

With new ways for household and personal care brands to build relationships and trust with the consumer, one thing is key – digital manufacturing and processing technologies will be at the forefront of change. In-line rheology measurement, a critical part of the manufacturing process, will be important for brands wanting to act quickly, allowing them to design flexible and adaptable production processes.

As digital transformation continues to aid the manufacturing industry, manufacturers must stay ahead of consumer trends to ensure they're not left behind.

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authors

Thomas Machin | tom.machin@streamsensing.com
Rob Travis | rob.travis@streamsensing.com

Stream Sensing Limited
Sunlight House | Quay Street | Manchester M3 3JZ
United Kingdom

DEUTSCHE GESELLSCHAFT DER PARFÜMEURE im SEPAWA® e.V.

DGP Spring meeting 2022

24th and 25th March 2022 in Barcelona, Spain

E. Diaz, A. Wilsch-Irrgang

After a two-year break, the spring conference of the "German Association of Perfumers in SEPAWA® e.V." took place again for the first time as a face-to-face event. Under the motto "*Scent and Inspiration in the City of Gaudi*", 34 participants met in Barcelona.

On Thursday, 24 March 2022, the lecture conference began at 12:00 with a joint lunch at the conference hotel Occidental Atenea Mar. Here, the participants had their first opportunity to exchange ideas in person.

Afterwards, **Edison Diaz**, President of the DGP, opened the proceedings with the activity report of the Board and Advisory Council on the past year.

In addition to the successful virtual Spring Conference 2021, the President reported on the update of the **DGP Chronicle**. The DGP was founded in 1979, the chronicle was revised as an anniversary edition for the 40th anniversary and presented in book form.

The **DGP 2022 sponsorship award** goes to **Akshita Joshi**, University of Dresden. Her work "*Neural Associations between well-being and odor perception*" was awarded. We hope to welcome Ms Joshi as a speaker at the SEPAWA® CONGRESS in October.

For the future, the DGP is planning the next spring conference from the 23rd to the 24th of March 2023 in Bad Dürkheim. There will be a study trip to Tunisia at the end of April 2023.

The first presentation was given by **Ana Ripoll Santos**, perfumer at Iberchem, Murcia, Spain. Under the title *"The Challenges of Natural Fragrances"*, she highlighted the great importance that fragrances have in our daily lives.

For critical consumers today, concepts such as organic, natural, vegan or degradable play a major role as a purchase criterion. Ms Ripoll explained in detail which ISO standards apply to natural fragrances, when a fragrance can be described as being of natural origin, and also how the difference between "organic" and "biological origin" is defined.

Provided that a fragrance meets the requirements of the ISO standard, it can be described as natural. However, this is not independently verified and there are no intrinsically prohibited ingredients within the standard. The criteria for natural ingredients are met by about 40% of the 1500 fragrances in a perfumer's palette.

The COSMOS standard for natural and organic cosmetics goes much further. COSMOS certifies compliance with its own standards, here only about 20 - 30 % of the standard palette can be used.

In view of the limitations mentioned above, it is indispensable for the development of a fragrance to define very precisely in the briefing which criteria the fragrance has to fulfil.

Ms Ripoll used four examples to show how fragrances with different claims can be designed. She also emphasised that "natural" is not synonymous with "sustainable" or "safe". In this respect, the industry also has a responsibility to do more

education to overcome a frequently encountered and misguided chemophobia.

In the discussion, it was asked whether the restrictions on the development of natural or COSMOS-certified fragrances do not impede the perfumer's creativity. Here, the opposite is the case, according to Ms Ripoll. One has to forget everything one has learned and very creatively develop completely new ones!

In the second presentation, **Ms Soizic Beaucourt**, perfumer at Eurofragance, Barcelona; introduced *"The Scents of the Gulf"*.

The Gulf region comprises the United Arab Emirates, the Kingdom of Saudi Arabia and Qatar. Fragrance has a tradition here that goes back thousands of years and is closely linked to religion. The Koran mentions 50 perfumes. Fragrances are also brought home from the pilgrimage to Mecca as gifts to the family.

In the Gulf States, eight times as much perfume is consumed per head as in Europe! Here, fragrance is inseparably linked to the expression of one's own personality. There are various forms of application for personal use: As pure fragrance oil as well as an alcoholic extract, without alcohol as a fragrance spray for the hair or as bakhoor (fragrance blocks), where fragrant substances are used on burning coals for incense.

Layering is very common in the Gulf States. This means that between 7 and 9 different fragrances are combined with each other.

Besides frankincense and myrrh, musk, sandalwood and oud are particularly characteristic of the Gulf States' fragrances. Oud or eaglewood is produced by Asian Aquilaria trees after infestation with a fungus in their wood. The pure oud essence from the distillation of eaglewood is one of the most valuable and expensive fragrance raw materials. One tree can be harvested after eight years at the earliest and yields only 32 ml of pure agarwood oil.

We had the opportunity to smell this rare and precious fragrance on its own, as well as two other classic scents: Mukhallat, based on rose, oud, saffron and sandalwood, and Musk Tahara or White Musk. With these, Soizic Beaucourt has transported us to the Orient.

After the coffee break, **Camila Tomas**, Vice President Innovation and Technology at Puig, Barcelona, continued the series of lectures with her expert talk *"Solving Key Pain Points in the Fragrance Industry"*. With brands such as Paco Rabanne, Carolina Herrera and Penhaligons, Puig is a major player in fine fragrances. For customers, the very large selection of fragrances makes it difficult to select the one that suits them best. Moreover, when trying out perfumes, the nose quickly tires.

Puig has developed an innovative system to recommend suitable fragrances based on customers' preferences.

One component is the internet-based profiler Wikiparfum. Registered users enter their personal preferences and receive fragrance suggestions. Wikiparfum contains a very extensive database of fragrances available on the market, which Puig has built up with its own scientifically collected and other independent data on the respective fragrances.

Another element is the AirParfum fragrance dispenser. It allows many perfumes to be smelled

without overloading the nose. The system is used in large perfumeries worldwide. From user feedback, it generates both customised suggestions for the customer and valuable customer data for the supplier.

An AirParfum device was presented to the participants in detail, and it could also be tried out for real.

We were impressed by the performance of the integrated system developed by Puig for a better understanding of customer profiles.

The lecture day was concluded by **Ada Parellada**, a well-known chef and owner of three restaurants in Barcelona. In her contribution *"Why Do We Eat the Way We Eat?"*, she passionately explained what criteria people really apply when choosing their food. It is not primarily the will to eat healthily in order to be fit and efficient. It is rather the food that is culturally known, what one has learned to prepare oneself, and finally, which food gives us positive feelings that play a big role. The emotionality of food closes the circle to smelling, which is also an emotional-sensual experience.

The programme continued after a short break: In a two-and-a-half-hour guided tour, the participants got to know the ancient city of Barcelona. We experienced an atmospheric tour that was packed with interesting information. It concluded at the restaurant El Cangrejo Loco, where we enjoyed the opportunity to talk to each other over excellent seafood paella.

On Friday morning, the second highlight of this year's spring conference: A visit to the company Lluch Essence SL, Barcelona. The owners **Eva and Sofia Lluch** welcomed our group and together with their team gave us a comprehensive insight into the performance of their company. Lluch is a third generation family business with an annual turnover of €100 million and 150 employees. The company distributes 4000 natural and synthetic raw materials for flavours and fragrances, sourced from 300 suppliers. Lluch is extensively certified and also offers contract manufacturing for fragrances and flavours. A sustainability report is produced annually and is available on the rightsupply website.

Together with **Ivan Borrego**, **Eva Lluch** informed about **Beauty Cluster Barcelona**, an association of 240 companies in the cosmetics and fragrance industry in Spain. The association has founded a Beauty Business School, where 15 students are currently receiving six-month basic training. It also supports an annual perfumery competition (Mouilette d'Argent). In 2021, there were 120 entries on the theme of "violets", with participants coming from more than 20 countries.

After a guided tour of the laboratory, administration, warehouse and mixing plants, Lluch surprised us with a delicious lunch, during which contacts were made and refreshed.

We would like to thank **Eva and Sofia Lluch** as well as **Thierry Bourrat** for the perfect organisation and the generous reception of our group!

After a long period of virtual exchange, all participants of the Spring Meeting 2022 were glad to be able to share experiences and adventures in person again and started their journey home with a wealth of new insights.

Dr. Edison Diaz
Dr. Anneliese Wilsch-Irrgang

Contact

Dr. Edison Diaz
Symrise AG
Mühlfeldstr. 1 37603 | Holzminden | Germany

Mediterranean Diet for Skin

Formulation-ID: 4085FOR1

Provital
Do Care

Phase	INCI / PCPC	Trade Name	Supplier	w/w %
A.1	**Olea Europaea Fruit Oil**	**Mediterranean Oil ECO**	**Provital**	**2.00**
A.2	**Prunus Amygdalus Dulcis Oil, Helianthus Annuus Seed Oil, Rosmarinus Officinalis Leaf Extract**	**Sweet almond oil**	**Provital**	**4.00**
A.3	**Vitis vinifera (Grape) seed oil**	**Grape seed oil**	**Provital**	**8.00**
A.4	"Hydrogenated Ethylhexyl Olivate and Hydrogenated Olive Oil Unsaponifiables"	Natura-Tec Plantsil™	Natura Tec	9.00
A.5	Polyglyceryl-3 Polyricinoleate	Imwitor® 600	IOI Chemicals	4.00
A.6	Silica Dimethyl Silylate	Makigel OL	Daito Kasei	2.00
A.7	Caprylic/Capric Triglyceride, Stearalkonium Hectorite, Propylene Carbonate	Miglyol® Gel B	IOI Chemicals	5.00
A.8	Helianthus Annuus Seed Cera, Ascorbyl Palmitate, Tocopherol, Helianthus Annuus Seed Oil	Kahlwax 6607 L MB	Kahlwax	0.50
B.1	Water (aqua)	-	-	57.50
B.2	Pentylene Glycol	-	-	2.00
B.3	Phenylpropanol, Propanediol, Caprylyl Glycol, Tocopherol	Sensiva® PA40	Schulke and Mayr	0.80
B.4	Magnesium sulfate	-	-	1.50
B.5	**Water,Glycerin, Jasminum Officinale (Jasmine) Flower Extract , Sodium Benzoate, Potassium Sorbate**	**Jasmine Extract HLG-MS**	**Provital**	**1.00**
B.6	**Propanediol, Aqua,Salvia Sclarea Extract, Sodium Benzoate, Potassium Sorbate**	**Xeradin™**	**Provital**	**2.00**
C.1	Fragrance (Parfum)	Dawn Jasmine	Luzi	0.20
C.2	Tocopherol	-	-	0.50

PROCEDURE:

1. In a clean beaker, weigh the ingredients of **phase A** from **A.1** to **A.5**
2. Then add **A.6** under stirring (dispersing blade) and stir at low speed until uniform
3. Then add **A.7** under homogenizer to activate it
4. Then add **A.8** and heat up the **phase A** to 80°C
5. In parallel prepare **phase B** mixing the ingredients **B.1** to **B.4** together and heating up to 80°C , add **B.5** and **B.6** just before making the emulsion
6. Add slowly **phase B** into **phase A** under high speed stiring , then homogenize for 2 min
7. Cool down under stirring to 30°C for the addition of **C.1** and **C.2**

CONTROL:

Appearance: Off White Thick Emulsion
pH: NA

Viscosity (20°C , 100 rpm, spindle 7)
Stability Test: 3 month at 50°C, 40°C and RT

DIRECTIONS:

Apply on every body and face dry area , where you need some extra reparation and let the skin absorbe it.

TARGET:

All skin types.

Bring Back My Curls Mist

Formulation-ID: 7345FOR2

Provital
Do Care

Phase	INCI / PCPC	Trade Name	Supplier	w/w %
A.1	Water (aqua)	-	-	60.85
A.2	**Aloe Barbadensis (Aloe Vera) Leaf Juice , Sodium Benzoate, Potassium Sorbate**	**Aloe Vera Gel ECO**	**Provital**	**20.00**
A.3	Hydrolyzed Corn Starch	MaizeCare™ Style Polymer	Dow Inc	3.00
A.4	Aqua, Lactic Acid, Propanediol, Citric Acid, Tartaric Acid, Gluconic Acid	Iscaguard IAF	Lemmel	1.50
A.5	Water (Aqua) , Sodium Hydroxide	-	-	1.30
A.6	1,3-Propanediol	-	-	3.00
A.7	**Water, Glycerin, Amaranthus Caudatus Seed Extract, Zea Mays (Corn) Starch, Benzyl Alcohol, Potassium Sorbate, Phenoxyethanol, Sodium Benzoate, Gluconolactone, Caprylyl Glycol, Phenethyl Alcohol, Calcium Gluconate, Tocopherol**	**AMA-PROT**	**Provital**	**2.00**
A.8	**Propanediol, Glycerin, Amaranthus Hypochondriacus Leaf Extract, Citric Acid**	**AMA-LEAF**	**Provital**	**2.00**
A.9	Water (Aqua), CI 17200 (0,1%)	D&C Red 33	Spectra	0.35
B.1	Dodecane	Parafol 12-97 RSPO-MB	Sasol	5.9998
B.2	CI 60725	Azul VI a la grasa	Sancolor	0.0002

PROCEDURE:

1. In a clean beaker , weigh **A.1** and **A.2** and mix them together
2. Under stirring add **A.3** , and mix until it is fully dispersed
3. Add the rest of the ingredients of **phase A** one by one
4. Prepare **B** mixing **B.1** and **B.2** together and then add it to batch under stirring
5. Fill the packgaging while stirring to ensure reproducibility of ratio water phase/oil phase between samples

CONTROL:

Appearance: Light Pink and Purple Biphasic Liquid
pH: 5.0-6.0

Viscosity (20°C): NA
Stability Test: 3 month at 50ºC, 40ºC and RT

DIRECTIONS:

Shake well and spray on your hair , until it is slighlty wet , then with your hands press it up to the roots to recreate some bounce.

TARGET:

Waxy , curly , coily hair types.

FORMULATIONS

SOFW's event preview

Did you know that SOFW not only publishes books and magazines but is also big in event organization?
We organize around 30 events a year in the home care, personal care, fragrance and, most recently, packaging industry.

Here you can find the upcoming events:

Date	Title and details
07. – 08.09.2022	**Workshop "Galenics, Liberation and Effect" by DGK e.V.** **Location:** Krefeld, Germany | **Format:** live | **Special Feature:** German language only **Website:** https://dgk-ev.de/dgk-termine/
15.09.2022 10:00 – 16:00	**SOFW eVENT "Personal Care | Skin Microbiome: How to manoeuvre throughthe metropolis of our skin!" by SOFW** **Format:** virtual | **Special Feature:** English language only **Website:** https://www.sofw.com/de/events/sofw-events/packaging-the-coconut-what-can-we-learn-from-packaging-by-nature
20.09.2022 10:00 – 11:30	**Expert Panel Microbiology "Microbiome" by DGK e.V.** **Format:** virtual | **Special Feature:** German language only **Website:** https://dgk-ev.de/dgk-termine/
28. – 29.09.2022	**Workshop "Practical training: Rheology in the cosmetic industry" by DGK e.V.** **Location:** Fulda, Germany | **Format:** live | **Special Feature:** German language only **Website:** https://dgk-ev.de/dgk-termine/
26.10.2022	**European Detergents Conference "Interface Interactions: Experiment & Modeling" by GDCh e.V.** **Location:** Berlin, Germany | **Format:** live **Website:** https://cosmetic-science-conference.com
26. – 27.10.2022	**Cosmetic Science Conference "Cosmetics 360 Degrees" by DGK e.V.** **Location:** Berlin, Germany | **Format:** live **Website:** https://cosmetic-science-conference.com
26. – 28.10.2022	**SEPAWA® CONGRESS by SEPAWA e.V.** **Location:** Berlin, Germany | **Format:** live **Website:** https://sepawa-congress.com
26. – 28.10.2022	**SEPAPack "Intelligent packaging for innovative home and personal care products" by SEPAWA e.V.** **Location:** Berlin, Germany | **Format:** live **Website:** https://sepapack.com
08. – 09.11.2022	**Workshop "Sunscreens" by DGK e.V.** **Location:** Witten, Germany | **Format:** live | **Special Feature:** German language only **Website:** https://dgk-ev.de/dgk-termine/
15.11.2022 10:00 – 11:30	**Expert Panel Microbiology "Cosmetics and microbiology: implementing ISO 29621 or what is a low risk product?" by DGK e.V.** **Format:** virtual | **Special Feature:** German language only **Website:** https://dgk-ev.de/dgk-termine/
08.12.2022 10:00 – 16:00	**SOFW eVENT "Personal Care | I have green hair: The natural way to care" by SOFW** **Format:** virtual | **Special Feature:** English language only **Website:** https://www.sofw.com/de/events/sofw-events/packaging-the-coconut-what-can-we-learn-from-packaging-by-nature

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Germany

Phone
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CSC
COSMETIC SCIENCE CONFERENCE
26–27 OCTOBER 2022

COSMETICS 360 DEGREES

The Cosmetic Science Conference (CSC) is the **key event for** all those interested in the latest trends in the cosmetic industry as well as in cutting edge cosmetic science – **formulators and researchers** alike.

During the two days, in **more than 20 presentations** we will focus on the topic "Cosmetics 360 Degrees".

The aim of this conference is to present the development of cosmetic sciences "holistically" (and around the focus of sustainability).

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