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IMPROVING SUNSCREEN PHOTOSTABILITY BY QUENCHING THE SINGLET EXCITED STATE

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Objective

The aim of the studies reported here is to demonstrate how a simple modification to the basic α -cyano- β,β -diphenyl acrylate molecular architecture changes its photophysical properties, enabling it to quench the singlet excited states of UV filters, thus increasing its efficacy as a quencher of photoinduced excited states and as a photostabilizer of the sunscreen filters Avobenzone (Butyl methoxydibenzoylmethane or BMDM) and Octyl methoxycinnamate (Octinoxate or OMC).

Introduction

Organic molecules consist of a positively charged nuclear framework surrounded by a negatively charged electron cloud. Most UV filters used in sunscreens are organic molecules that absorb solar UV radiation (UVR). One molecule absorbs one discrete packet of UVR called a photon. The energy in the photon initially causes an electron in the negatively charged cloud to increase its energy and distance from the nuclear framework.¹ The initial event takes place in approximately 10^{-15} second². The initial state, called the singlet excited state, is short-lived: typically persisting for 10^{-12} - 10^{-7} second³. The molecule may dissipate its excited state energy and return to its pre-absorbance state -- the ground state -- by emitting a lower energy photon, an event known as fluorescence. Commonly, only a small number of molecules fluoresce. Most rapidly decay to a less energetic and relatively long-lived excited state, the triplet excited state. The long lifetime of the triplet excited state (10^{-6} - 10^{-1} second⁴) and its biradical character⁵ make the molecule vulnerable to a number of chemical reactions that may be destructive to its ability to absorb another photon⁶. In this way, BMDM is particularly vulnerable to destructive chemical reactions. BMDM in the triplet excited state also reacts chemically with OMC,⁷ which is destructive to the ability of both compounds to absorb UVR.

Some compounds such as octocrylene have the ability to limit or "quench" BMDM's triplet excited state, returning it to the ground state and reducing or eliminating destructive chemical reactions. Compounds possessing this ability are known to sunscreen formulators as "photostabilizers" or "triplet quenchers." Octocrylene and a few other compounds are effective at high concentrations, but less effective at low concentrations and when BMDM and OMC are combined.

Theoretically, a photostabilizer that is capable of quenching the singlet excited states of photolabile UV filters, such as BMDM, would be effective at lower concentrations and would improve the performance of formulations that combine BMDM and OMC. One of the many compounds synthesized added a methoxy group to one of the phenyls of cyano diphenyl propenoic acid to produce a new compound named "methoxycrylene." Tests were performed on the 2-ethylhexyl ester (INCI: Ethylhexyl Methoxycrylene).

Qualitative means were used to test ethylhexyl methoxycrylene's ability to quench the visible fluorescence of BMDM, OMC, and other fluorescing compounds. Sophisticated means were used to measure BMDM fluorescence -- spectra, quantum yield, and lifetime -- and to test methoxycrylene and Octocrylene for their ability to operate on BMDM's singlet excited state. Several *in vitro* performance and dose response studies were conducted by irradiating thin films that contained varying concentrations of methoxycrylene with BMDM alone and with other photoactive compounds. Finally, creams and lotions were prepared with methoxycrylene and BMDM and other UV filters and clinical studies were done to determine both SPF and UVA protection factors.

Brief Description of Methods

Fluorescence spectra were measured on a Fluorolog-3 (Jobin Yvon). Qualitative fluorescence quenching experiments were done by spotting solutions on thin layer chromatography plates and illuminating them with long wave (peak 365 nm) UVR. Fluorescence lifetimes and quenching measurements were determined on a streak scope (Hamamatsu model C4334) with excitation provided by a 150 femtosecond pulse width Ti:Sapphire laser. In vitro performance and dose response measurements were made on a UV 2000S Transmittance Analyzer (Labsphere). PMMA plates (Helioscreen) and Vitro-skin (IMS) were the substrates. UVR was provided by a Model 16S Solar Simulator (Solar Light Company). Clinical SPF and UVA protection factors were determined by Consumer Product Testing Co., Fairfield, NJ using FDA and JCIA (PPD) protocols respectively.

Selected Results

Ethylhexyl methoxycrylene quenches the visible fluorescence of BMDM; octocrylene does not. BMDM fluoresces with a quantum yield of .016 and a lifetime of 1.3×10^{-11} second (13 picoseconds). Methoxycrylene quenches BMDM's singlet excited state; octocrylene does not. A thin film containing 3% BMDM and 3% ethylhexyl methoxycrylene (3% *octocrylene*) retained 90.4% (85%) of its UVA absorbance and 97.7% (92.9%) of its UVB absorbance after 25 MED. A thin film on a substrate containing 7.5% OMC, 3% BMDM, and 5% ethylhexyl methoxycrylene (5% *octocrylene*) retained 8.3% (63.7%) of UVA absorbance and 89.8% (82.7%) of UVB absorbance after 25 MED. A sunscreen formulation containing 7.5% OMC, 3% BMDM, and 7% ethylhexyl methoxycrylene achieved SPF 34 and PFA 18.19 *in vivo*.

Conclusions

1. BMDM's singlet excited state is extremely short-lived at 13 picoseconds. Even so, adding a methoxy to the basic cyanodiphenyl acrylate framework enables the resulting methoxycrylene compound to quench BMDM's singlet excited state.
2. At equal weight concentrations, ethylhexyl methoxycrylene is more effective than octocrylene at preserving BMDM's UVA and UVB absorbance in the absence of OMC.
3. Ethylhexyl methoxycrylene is greatly superior to octocrylene at preserving BMDM's UVA and UVB absorbance in the presence of OMC.
4. Formulations containing BMDM and OMC can achieve high UVA protection factors when photostabilized with ethylhexyl methoxycrylene.

¹ Turro NJ. Modern molecular photochemistry. Menlo Park: Benjamin/Cummings, 1978; p. 3

² *Ibid* p. 7

³ *Ibid* p. 7, p. 90, p. 352

⁴ *Ibid*

⁵ *Ibid* p. 229

⁶ *Ibid* p. 329

⁷ D Dondi, A Albini, and N Serpone, Interactions between different solar UVB/UVA filters contained in commercial suncreams and consequent loss of UV protection, *Photochem. Photobiol. Sci.*, 2006, **5**, 835-843

REDUCTION OF DERMAL UV FILTER UPTAKE IN SKIN AND BOOSTING *IN VIVO* SPF/UVA USING ENCAPSULATE TECHNOLOGY AND FOCUSED FORMULATION STRATEGY

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INTRODUCTION:

Protecting the skin from the adverse effects of UV radiation is a major goal in skin care. Can adequate UVA/B protection be achieved with minimal dermal penetration of organic sunscreen? A micro-encapsulation technology entrapping organic sunscreen in a sol-gel silica glass was investigated to answer this question. The sol-gel process permits encapsulated organic oil-soluble sunscreens to be formulated into the aqueous phase of an emulsion. Encapsulation also permits incompatible ingredients such as Avobenzone (BMDDBM) and Octinoxate (OMC) to be formulated together. These two properties enable the formulator to create a wider range of sunscreen products with low allergy potential using an inert capsule.

METHODOLOGY:

***In vitro* percutaneous absorption:** Porcine skin (10 week old male) was obtained for this study. Skin membranes were prepared by removing all subcutaneous fat and part of the dermal tissue. The thickness of each membrane was determined to be on average 0.642 \pm 0.046 mm as determined by a digimatic micrometer (Mitutoyo Corporation, Japan). Sterile glass rings with an internal area of 1.5 cm² were glued to the epidermal side of the membranes using cyanoacrylate-based glue. The membranes were transferred into six-well plates on a Netwell insert allowing contact of the receptor fluid to the dermal side of the membranes leaving the stratum corneum exposed to air. The six-well plates were placed in a humidified incubator at 32 $^{\circ}$ C. Receptor fluid consisting of DMEM culture medium supplemented with EGF (10 μ g/l), hydrocortisone (400 μ g/l), gentamicin (50mg/l), and fetal calf serum (10%v/v). Experiments were performed with a permeability coefficient (Kp) of less than 2.5 x 10⁻³ cm/h for titrated water. Test material was applied to the membranes by glass rods. Samples of receptor fluid were collected at 1, 2, 4, 6, 8, 20, 22, 24 hours after application while the same amount was restored by adding fresh receptor fluid. Oil-in-water emulsions formulated with and without UV filter (OMC) were used as controls and compared to another emulsion containing encapsulated OMC. Each membrane was tape-stripped 10 times. The remaining epidermis and dermis was isolated by heat separation. All samples (receptor fluid, skin surface, stratum corneum strips, epidermis and dermis) were stored separately for HPLC analysis.

***In vivo* photo-acoustic stratum corneum uptake:** Dermal uptake was determined by monitoring the amplitude of heat response when test formulation is applied to skin. Energy from a light source is transferred to a sample via light absorption and partially re-emitted as heat. The infrared wavelengths are transformed into a photo-acoustic signal detected by a sensor. The study was performed at two wavelengths of light, 266 nm to monitor the OMC (light is absorbed mostly by OMC) and 355 nm to observe formulation effects (light mostly absorbed by the skin background). Energies of light irradiation were kept below erythema levels at 0.8 mJ/cm² for 355 nm and 0.4 mJ/cm² for 266 nm.

***Ex vivo* epidermal uptake:** Human female abdominal skin was used to heat-separate the epidermis from the dermis. Epidermal membranes were mounted on a Franze-type diffusion cell. Each formulation applied to 3 cells, non-occluded, with a finite dose concentration of 5 mg/cm². A formulation with free OMC was compared to a similar formulation with encapsulated OMC after a 6-hour period. HPLC was used to evaluate OMC concentration.

***In vivo* SPF boost:** SPF measurements were conducted using 5 volunteers under standard test protocols. Two formulations, one containing free OMC and the other containing encapsulated OMC were compared for SPF value. Another study compared formulations with the encapsulated sunscreen added in the water phase compared to non-encapsulated material added to the oil phase.

Photo stability of OMC/BMDDBM combination: An emulsion containing free OMC/BMDDBM was compared to a similar emulsion with encapsulated OMC/BMDDBM. Each emulsion was spread onto frosted glass plates (1 mg/cm²) and irradiated with a Suntest instrument at 1 MED for 15 minutes. The control plate at 0 min was analyzed at the end of the test in order to evaluate the potential oxidation process due to air. An HPLC was used for analysis.

Prevention of singlet oxygen formation ($^1\text{O}_2$): Free OMC may trigger formation of $^1\text{O}_2$. Formation of $^1\text{O}_2$ was investigated by published methods (1,2) Using a photosensitive dye, methylene blue and histidine color fading representing a $^1\text{O}_2$ reaction with reactive nitrogen species was determined spectrophotometrically.

RESULTS:

In vitro percutaneous absorption: A considerable reduction in total dermal absorption was observed in membranes treated with encapsulated sunscreen. Roughly 92.87% of the applied dose of the free sunscreen and 96.74% of encapsulated sunscreen remained on the skin surface. With respect to total skin absorption, 70% less sunscreen was measured in receptor fluid + epidermis + dermis, (Figure 1). **Photo-acoustic uptake-** A high photo-acoustic response of encapsulated OMC at 90-120 min confirms efficient UV indicates encapsulate sunscreen remains on the skin (Figure 2). **Epidermal uptake-** a 3-fold reduction in uptake was measured in the encapsulated treated material. **SPF boost-** an increase of approximately 5 SPF units was measured. **Photo stability-** after 120 min of irradiation 70% of the BMDBM was observed intact in the encapsulate compared to 20% in the non-encapsulated material (Figure 3). **Singlet oxygen formation-** encapsulated UV filter did not show evidence of singlet oxygen formation (Figure 4).

CONCLUSIONS:

The sol-gel encapsulation process provides an enhanced margin of safety due to reduced dermal penetration of sunscreen. The ability to formulate OMC with Avobenzone provides for a broader range of effective sunscreen products.

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Figure 1:

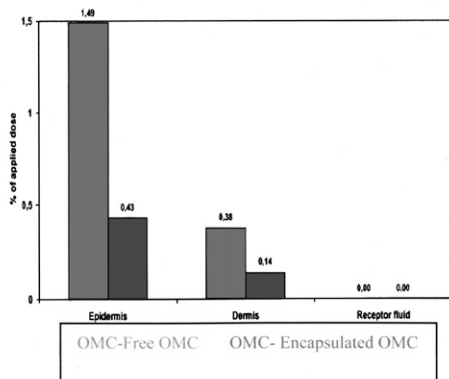


Figure 2:

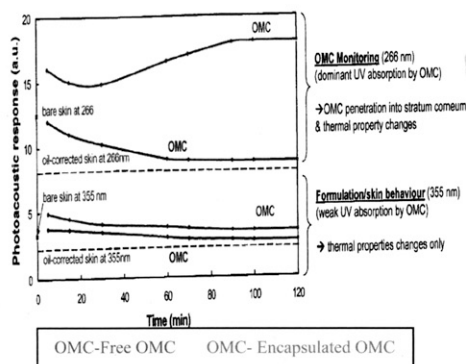


Figure 3:

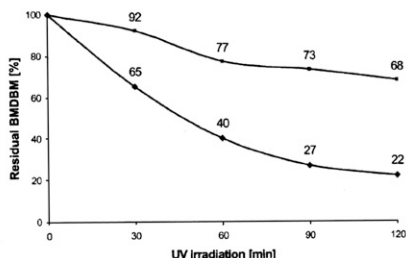
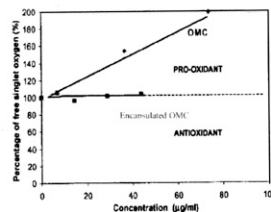


Figure 4:



Singlet oxygen formation of free OMC or Encapsulated OMC

NON-NANO ZINC OXIDE

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Introduction

Inorganic UV filters, Titanium Dioxide and Zinc Oxide, have been used in sunscreen products since mid 1980's^(1,2) because of their efficacy and safety record. The control of their particle size is known to be a key element in obtaining optimum UV attenuation and a transparency acceptable for cosmetic use. Since transparency is an important characteristic for inorganic sunscreens, we decided to focus our study on zinc oxide, because it has a lower refractive index than titanium dioxide and thus larger particle size pigments could be considered.

In the past few years, arguments have been made by consumer groups and environmental organizations about the safety of nanomaterials, defined as particles smaller than 100 nanometers. This has led the scientific community and governmental agencies to re-evaluate the rules regulating the use of these nanomaterials, including inorganic UV filters for cosmetic applications. Earlier this year, the European Parliament has approved a new legislation, which includes a definition and conditions of use for nanomaterials. However, as of yet, no official method for measuring particle size has been published.

In the course of this work, we will discuss the merits and drawbacks of the most popular measurement methods and their capacity to give us answers about claims on particle size. Then, we will present several 'Non-Nano' Zinc Oxide and evaluate their efficacy as UV filters.

Size measurement

1. Particle size :

In March 2009, the EU Parliament have approved a modification in the Cosmetic Directive⁽³⁾, which includes a definition of a nanomaterial as "an insoluble or bio-resistant and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm."

When Zinc Oxide is manufactured, the process defines a crystal size, often referred to as 'primary particle size' (PPS). However, when supplied as a powder, particles are agglomerated. The size of these agglomerates have to be reduced in order for the pigments to perform efficiently as a sunscreen : the size of the resulting aggregates (PS) depends on the primary particle size and on the conditions of the size reduction process. It is the size of these aggregates which gives its optical properties and UV attenuation capacity to a particle.

It is difficult to say, based on the EU definition, which size, PPS or PS, has to be taken into account to decide if a particular product contains nanomaterials or not. Furthermore, depending on the method chosen, either the primary particle size or the size of aggregates will be measured. It is therefore of great importance to study the different methods available when producers or formulators need to report the presence or the absence of nanomaterials in a product.

2. Significance of the size measurement :

Based on the know theory of light scattering, manufacturers and formulators have learned to manipulate the particle size of the inorganic UV filters to obtain the attenuation of a specific UV light range. To achieve this goal, the mean size is the only value necessary.

With the new regulations, and the need to report the presence of even small percentages of nanomaterials in a product, accurate descriptions of the particle size distributions have become necessary. However, most size measurement methods are not reliable for this type of measurement and it is therefore impossible to accurately determine the size of the smallest particle in the distribution : a d0 has no meaning in statistics.

Results can also be expressed differently, depending on the instrument used. Of particular importance is the way the results are averaged, either by length or by volume. Depending on how they are expressed, the results for the same product could be different.

Sample preparation should also be carefully evaluated. If the sample is dried, or on the contrary diluted, agglomeration of the particles can occur and give rise to a measurement larger than the actual value. Finally,

depending on their polarity, some solvents can disperse inorganic UV filters efficiently, when others will promote agglomeration. When the UV filters are treated to change their properties, this behavior can change.

3. Size measurement methods :

They are two different types of methods commonly used and capable of measuring the size of small particles : electron microscopy followed by an image analysis or the measure of a global characteristic of a suspension, particle size is then derived from this parameter.

3.1. *Electron microscopy and image analysis* :

Electron Microscopy generates an image of the product analyzed and therefore appears to be an easy way to calculate a size for the sample, knowing the image magnification.

Depending on the type of technique (scanning – SEM – or transmission –TEM) used and on the sample preparation, it is possible to measure either the primary particle size or the size of aggregates / agglomerates of a powder.

However, as with any other techniques, Electron Microscopy has its limitations and biases.

- Statistical / sampling : because of the need to know if any particle is a nano-particle, therefore to get a good description of the size distribution, it is very important to measure a great number of particles, which is not possible with Electron Microscopy. The number of measures commonly made is usually sufficient to obtain a mean value with acceptable accuracy. ISO 13322-1 ⁽⁴⁾ gives guidelines on the measurement of particle size obtained from image analysis. Even with narrow size distributions, a number of particles in excess of 50,000 should be measured, according to these guidelines, to obtain reliable values if the description of the size distribution is required.
- Geometry : particles are obviously three dimensional objects, and the new EU regulation states that all three dimensions have to be measured. However, the analysis is made on a two dimensions image : if the particles are non-spherical, this could introduce a bias.
- Sample preparation : because the samples need to be dried, important agglomeration occurs. Especially for TEM, it implies that part of the sample cannot be measured and analysis has to be limited to portion of the image where particles do not overlap. This raises the question of a selection of particles that might not be representative of the whole sample.
- Ease of use : even if Electron Microscopy has become more easily available, it is still not a technique that can be routinely used for size measurements.

3.2. *Dynamic Light Scattering (DLS)* :

This technique, also called Photon Correlation Spectroscopy, measures the coefficient of diffusion of particles moving (by Brownian motion) in a solvent. The size of the sample is then derived from this coefficient using the Stokes-Einstein equation. It is probably the most widely used method for measuring small size particles, because of its ease of use and its precision for mean values ⁽⁵⁾. However, it cannot accurately be used to represent a size distribution and therefore is of little use for determining if a sample contains a small amount of nanoparticles.

3.3. *Laser Diffraction (LD)* :

Also called 'Static' Light Scattering, Laser Diffraction instruments analyze the light scattering properties of a suspension of particles and convert it in particle size ⁽⁶⁾. Laser Diffraction gives a reliable description of the size distribution; but it measures the size of aggregates in a solvent and cannot be used with highly concentrated dispersions. It is however the best and easier to use method.

3.4. *Acoustic attenuation spectroscopy* :

Because particles also scatter sound, this method uses ultrasound for measuring the size of particles that are dispersed in a fluid. Even though the theory has been known for a very long time, the applications in the area of size measurement are relatively new ⁽⁷⁾, and this technique has therefore limited background. However, it can be used with no dilution or other sample preparation, making it a promising technique.

Manufacturers of Zinc Oxide tend to use Electron Microscopy to measure the size of their material. However, light scattering techniques (DLS or LD) are favored by most downstream users. Therefore, in the second part of this study, we will present examples of Zinc Oxides that can claim 'Non-Nano' status, measured either by Electron Microscopy or by Laser Diffraction.

UV Attenuation Grade ZnO of Large Particle Size

Most UV attenuation grade ZnO have a primary particle size smaller than 100nm. We have analyzed by Electron Microscopy the size distribution of Pigmentary (conforming to the USP specifications) and large size ZnO available on the market, to see if they could be called 'Non-Nano' grades.

Sample	SSA (m ² /g)	Particle size range (nm)
Commercial A	4.0 – 5.5	50 – 1200
Commercial B	3.0 – 5.0	50 – 720
Commercial C	5.0 – 7.0	50 – 1600

All of them have very large size distribution, with a significant population of particles under 100 nm, and the very large particles limit their UV attenuation performance and their cosmetic acceptance (whitening).

We have then studied a new ZnO (ZnO-C) recently developed by Sumitomo Osaka Cement. The primary particles of ZnO-C are in the range of 100 - 400 nm. Electron Microscopy studies show that no particles are smaller than 100nm, making this grade a real 'Non-Nano' material.



SEM picture of ZnO-C

In order to test its UV attenuation performances, ZnO-C was dispersed in C12-15 alkyl benzoate and formulated into a W/O formulation. It was then compared with a 35 nm. ZnO pigment which was prepared using the same conditions..

	Zno-C	Ultrafine ZnO
InVivo SPF	12.1	~ 15 - 20
In-Vivo PFA	5.52	~ 7.5 - 8
In-Vitro SPF	5.68	NA
UVA ratio	0.88	~ 0.6 – 0.85
Critical Wavelength	381 nm	~ 370 – 375 nm

The in-vivo results show that ZnO-C has lower UV attenuation than Ultrafine ZnO. However, even if the size of ZnO-C is large, it is still quite effective. It also delivered a critical wavelength over 380 nm, which can be rarely achieved with other grades of ZnO.

Similar results were found for the whitening of the skin when lotion was applied. ZnO-C, due to its large primary particle size, shows a small increase in whitening as compared with smaller grades.

It is therefore possible to use this new grade ZnO for sunscreen products, without having to report the presence of nanomaterials.

Surface treatments promoting large aggregates

ZnO are often surface treated to be hydrophobic for good wetting in a formula and pleasant skin feel. We have evaluated several hydrophobic treatments and found out the a Jojoba ester treatment is able to cause the primary particles to form aggregates and meet the non-nano criteria

In this study, the powders are dispersed in solvent and sonicated internally before measurement by the Laser Diffraction method. Ethanol then hexane were used to represent polar and non-polar media.

When a 5% Jojoba Ester coating was applied to a 20 nm. (PPS) Attenuation grade ZnO, the size distribution shown no particles bellow 100nm. Jojoba esters seems to act as a binder for the primary particles. Even when dispersed and milled, the sizes measured were still all over 100 nm.

This powder was dispersed in esters or vegetable oil and formulated into sunscreen lotions. SPF and PFA were tested in-vivo on 3 panelists. The results are listed below and indicate that the UV attenuation power of this non-nano ZnO is equivalent to that of their nano counterparts. In addition, their transparency on skin was as good.

	Jojoba Ester treated ZnO
Formula Type	(W/O)
PS in disp. (nm)	Above 100 nm
Active (%)	15
SPF	22.4
PFA	8.67

Summary

Concerns over safety of nano materials and changes in regulations have led to the need of inorganic UV filters for which a 'Non-Nano' (absence of particles bellow 100nm) label claim can be made. The analytical method used to measure the particle size distribution is therefore of great importance. We have review numerous methods and shown that, depending on the size that one wants to report, either Electron Microscopy coupled with Image Analysis or Laser Diffraction are the method of choice.

We have show that a new grade of large particle size ZnO, because of its relatively narrow distribution, can be used in sunscreens with great benefits. Another grade, after being treated with Jojoba Esters, also shown to be 'Non-Nano', this time when measured with Laser Diffraction.

Since no official size measurement method has been yet published, and without knowing how the industry and the regulation agencies will follow the new EU Parliament rules on nanomaterials in other parts of the world, companies which commercialize sunscreen products will have to decide which measurement method would be acceptable and how they want to report the presence or absence of nanoparticles. The example presented here show that it is possible to formulate efficient sunscreens while claiming a 'Non-Nano' product.

Literature

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- 4- ISO 13322-1:2004 - Particle size analysis -- Image analysis methods -- Part 1: Static image analysis methods
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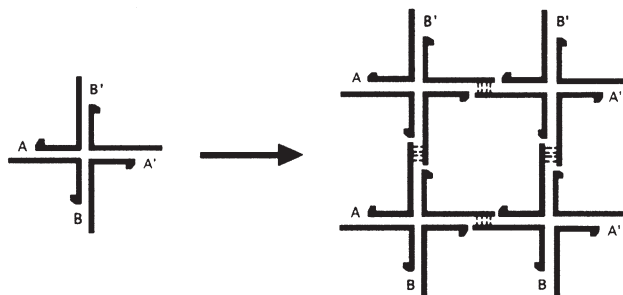
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DNA: NOT MERELY THE SECRET OF LIFE

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DNA is well-known as the genetic material of living organisms. Its most prominent feature is that it contains information that enables it to replicate itself. This information is contained in the well-known Watson-Crick base pairing interactions, adenine with thymine and guanine with cytosine. The double helical structure that results from this complementarity has become a cultural icon of our era. To produce species more diverse than the DNA double helix, we use the notion of reciprocal exchange, which leads to branched molecules. The topologies of these species are readily programmed through sequence selection; in many cases, it is also possible to program their structures. Branched species can be connected to one another using the same interactions that genetic engineers use to produce their constructs, cohesion by molecules tailed in complementary single-stranded overhangs, known as 'sticky ends.' Such sticky-ended cohesion is used to produce N-connected objects and lattices [1]. This notion is shown in the drawing, which shows cohesion between sticky-ended branched species.



Structural DNA nanotechnology is based on using stable branched DNA motifs, like the 4-arm Holliday junction, or related structures, such as double crossover (DX), triple crossover (TX), and paranemic crossover (PX) motifs. The design of stable branched molecules is based on the notion of minimized sequence symmetry [2]. We have been working since the early 1980's to combine these DNA motifs to produce target species. From branched junctions, we have used ligation to construct DNA stick-polyhedra and topological targets, such as Borromean rings. Branched junctions with up to 12 arms have been produced. We have also built DNA nanotubes with lateral interactions.

Nanorobotics is a key area of application. PX DNA has been used to produce a robust 2-state sequence-dependent device that changes states by varied hybridization topology. We have used this device to make a translational machine that prototypes the simplest features of the ribosome. Two

protein-activated devices have been developed that can measure the ability of the protein to do work, and bipedal walkers, both clocked and autonomous have been built. We have also built a robust 3-state device that includes another state, corresponding to a contraction.

A central goal of DNA nanotechnology is the self-assembly of periodic matter. We have constructed 2-dimensional DNA arrays from many different motifs. We can produce specific designed patterns visible in the AFM. We can change the patterns by changing the components, and by modification after assembly. Recently, we have used DNA scaffolding to organize active DNA components, as well as other materials. Active DNA components include DNAzymes and DNA nanomechanical devices; both are active when incorporated in 2D DNA lattices. We have used pairs of PX-based devices to capture a variety of different targets. Multi-tile DNA arrays have also been used to organize gold nanoparticles in specific arrangements.

Recently, we have self-assembled a 3D crystalline array and have solved its crystal structure to 4 Å resolution, using traditional unbiased crystallographic methods. Eight other crystals have been designed following the same principles of sticky-ended cohesion. Thus, structural DNA nanotechnology has fulfilled its initial goal of controlling the structure of matter in three dimensions. A new era in nanoscale control is just beginning.

This research has been supported by the National Institute of General Medical Sciences, the National Science Foundation, the Army Research Office, the Office of Naval Research and the W.M. Keck Foundation.

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ADVANCED REGRESSION MODELS FOR SOAP REFORMULATION

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A. OBJECTIVE

We investigate what role regression modelling can play in optimizing tradeoffs among combinations of base oils available for soap formulation. These oils include tallow, palm oil, palm kernel oil, and coconut oil. The formulator typically performs “ingredient substitution,” i.e., swapping one or more ingredient in or out of the formulation. The formulator’s choice “...will affect the quality of the final soap. For example, short-chain fatty acids are more soluble; they yield soaps that are more foaming, more irritating to skin, and that wear faster. A compromise must be found to obtain a mixture which satisfies the desired performance/cost criteria.” [1]

B. METHODOLOGY

Simple models are capable of predicting the effects of varying concentrations of ingredients on the performance of a formulation. When the ingredients vary between different formulations, however, these models become unreliable. The reason is apparent from examining the structure of the data, an example of which is presented below in Table 1:

	Coconut oil	Palm kernel oil	Tallow	Palm oil	Remainder*
Form 1	7	0	63	0	30
Form 2	14	0	56	0	30
Form 4	0	7	63	0	30
Form 5	0	14	56	0	30
Form 7	7	0	0	63	30
Form 8	14	0	0	56	30
Form 10	0	7	0	63	30
Form 11	0	14	0	56	30

*(Polyethylene glycol, Glycerol, Free Fatty Acids, Water, salts)

Table 1. Selected transparent soap formulations, based on the starting formulation given in Table 8.4 of [1]. The base oils are substituted in combinations similar to the 80 tallow: 20 coconut in the starting formulation.

A large number of the concentrations in the above table are zero, which adversely affects the simple models referred to above (for example linear regression models which use the concentrations as the descriptor variables). The typical approach to modelling ingredient-substitution formulation data is to keep the data as-is, but revert to a categorical modelling method, which effectively replaces the ingredients being substituted with a single variable taking on discrete values (e.g. the ingredient name). This approach can result in a model which is adequate from a statistical sense, but which is lacking in predictive power and scientific understanding. What is needed is to build models which allow the formulator to understand why substituting a particular ingredient has either improved or degraded formulation performance, and not simply capture the fact that this has happened, as categorical models do. These models simply don’t contain enough information to answer the formulator’s question “what was it about that particular ingredient which worked?”

Our method leverages ingredient information (both composition and property information), together with mixing rules in creating reliable regression approaches that are capable of answering these important questions. We have created software which implements this approach in a Web Services environment, making use of the Simple Object Access Protocol (SOAP).

The use of this particular software methodology is important, in that it puts the focus of the modelling not so much on the use of a statistical method once the data is gathered, but on the gathering of the data in the first place. Too much focus is sometimes given to the former activity, and too little to the latter. We believe that more R&D value can be obtained from modelling if this propensity is reversed. Care

must be taken, however, in doing this, as the data gathering work can simply be pushed onto the formulator, with adverse results. Ingredient information is typically dispersed throughout an organization. It is no longer adequate to expect the formulator to be responsible for seeking out and gathering this information in a form suitable for modelling. Such approaches do nothing to minimize the costs of hidden or tacit knowledge.

With suitable software tools, ingredient information from diverse sources (e.g., stored in spreadsheets, or a database) can be simply made available as a Web Service. In our case, our ingredient database contains both property and composition information about the base oils used in transparent soap formulation. The properties include titre, iodine value, saponification value, Reichert value and Polenske value. The compositional information includes a breakdown of concentrations of short-chain fatty acids, similar to the data presented in Table 8.1 of [1].

A mixing rule is simply a way of combining the property or compositional information for each ingredient into a value for the entire formulation [2]. For example, multiplying the saponification value for each ingredient by that ingredient's concentration, and summing over ingredients, gives an average saponification value for the formulation. More complex mixing rules may also be constructed, but typically the simplest ones are adequate.

C. RESULTS

Using our approach, we have transformed data on 12 transparent soap formulations into a form where simple regression methods can successfully analyze it. Table 2 below gives the same formulation data as in Table 1, but now transformed using mixing rules:

	Avg Sap value Value	Avg Iodine Number	Avg C14	Avg C16	Avg C16 1=
Form 1	141.47	30.205	3.4020	16.786	2.9680
Form 2	145.74	27.510	4.4240	15.652	2.6460
Form 4	140.77	30.905	3.2760	16.730	2.9750
Form 5	144.34	28.910	4.1720	15.540	2.6600
Form 7	145.88	33.985	2.1420	36.505	0.25900
Form 8	149.66	30.870	3.3040	33.180	0.23800
Form 10	145.18	34.685	2.0160	36.449	0.26600
Form 11	148.26	32.270	3.0520	33.068	0.25200

Table 2. Selected transparent soap formulations, represented using mixing rules. These replace ingredient concentrations with either averaged properties (the first two columns), or with averaged concentrations of constituents of the original ingredients (columns 3 through 5). In this case the constituents are the short-chain fatty acids known to determine soap performance properties such as skin irritation, wear, and foaming.

This results in models for skin irritancy that have good predictive power. The models are thus well-suited to being used in a formulation optimization process (e.g. involving a numerical optimizer, as discussed in [2]). Such an optimization process would involve finding the value of the ingredient properties or compositions which would optimize formulation performance, and would furthermore drive an understanding of what makes an optimal formulation.

D. CONCLUSION

Our approach produces predictive models which can be used not simply to understand existing processes, but to discover new ones. This distinguishes it from approaches which treat data using categorical modelling methods.

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EMPLOYING DNA TO AUTHENTICATE THE BOTANICAL ORIGIN OF INGREDIENTS USED IN COSMETICS AND TOILETRIES

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A. OBJECTIVE

The marketing of most cosmetic products emphasizes the inclusion of naturally derived ingredients. We have developed SigNature DNA and BioMaterial GenoTyping™ methodologies to authenticate personal care products and botanically-sourced ingredients. Cosmetics, biotherapeutics, nutraceuticals, natural foods, wines and fermented alcohols, paper and natural textiles are just some of the products that can be safeguarded with this technology. The DNA from botanical genomes can also be used to tag decorative inks, labels, shrink wrap, extruded plastics and finished crèmes and lotions. This presentation will provide recent findings on 1) the DNA techniques utilized to authenticate the DNA in a botanically derived ingredient, 2) methods used to DNA-tag components and products, 3) methods used to authenticate DNA-tagged materials, 4) the value of DNA toward enhancing quality control safety and efficacy of cosmetics, and 5) the use of DNA tags to identify originals, interdict counterfeits and prevent diversion of cosmetic products.

B. SAMPLES

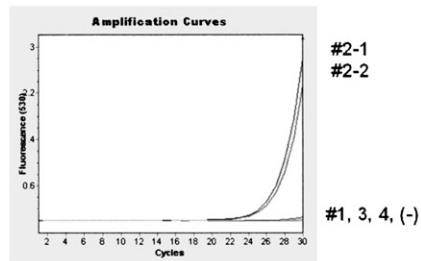
- I. Botanical DNA embedded into a moisturizing cream
- II. Botanical DNA embedded into ink that is applied onto a glass cosmetic package

C. METHODOLOGY

- I. **Botanical DNA embedment and recovery from moisturizing cream**
 - 50 nanograms plant DNA suspended in 100 µl PBS buffer
 - Blended into 60 ml moisturizing cream
 - 3 blanks and 1 DNA sample used for DNA recovery
 - Conventional and nested PCR methodologies are employed to establish a forensic proof of authentication.
- II. **Botanical DNA embedment and recovery from ink that is applied onto glass cosmetic package**
 - DNA was added to ink and tested at room temperature and at 300 degrees Celsius
 - DNA was added to six different inks. 1 ml of hardener and 2 ml of thinner was added to each ink to reconstitute the working ink formulations and mixed well.
 - 20 µl of each ink were used for DNA extraction
 - 4 µl of each extract were then used for DNA amplification
 - Conventional and nested PCR methodologies are employed to establish a forensic proof of authentication. The PCR amplicons were run by agarose gel electrophoresis and visualized under UV illumination

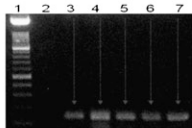
D. RESULTS

- I. **Botanical DNA embedment and recovery from moisturizing cream**
 - Positive DNA authentication

Figure1.**Detection of DNA from Moisturizing Cream**

- II. **Botanical DNA embedment and recovery from ink that is applied onto glass cosmetic package**

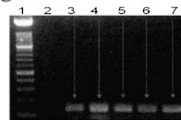
- Positive DNA authentication at room temperature
- Positive DNA authentication at 300 degrees celcius

Fig 2. DNA in on Package at RT

1.5% Agarose Gel

1. DNA Ladder
2. Negative Control
3. Positive Control
4. Ink 1
5. Ink 2
6. Ink 3
7. Ink 4

Botanical DNA was
successfully detected in all
inks

Fig 3. DNA ink on Package at 300°C

1.5% Agarose Gel

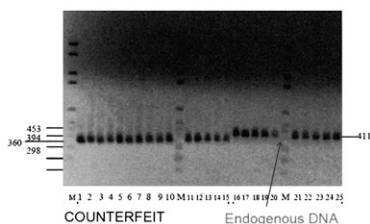
1. DNA Ladder
2. Negative Control
3. Positive Control
4. Ink 1
5. Ink 2
6. Ink 3
7. Ink 4

Botanical DNA was
successfully detected in all
inks

D. CONCLUSION

Our results indicate that DNA was positively and consistently identified in the cosmetic formulation itself, as well as on the cosmetic package. We also have developed a new methodology that involves identify the “genotype” of a particular ingredient in a cosmetic..

Distinguishing Genuine Cosmetic Product from a Counterfeit



CHARACTERIZATION OF THE COMPLEXION RADIANCE BY SENSORY AND INSTRUMENTAL ANALYSIS: DIFFERENCES OBSERVED BETWEEN POPULATIONS' CLASSES

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R&D Department, Silab, France

Introduction:

The complexion radiance is difficult to analyze and to describe: it reflects our own general state once physical and psychological according to our emotional state, our state of fatigue or more, the impact of environment on the skin... The main factors that are involved in the complexion radiance are the colour, the texture, the luminosity, the transparency and microcirculation which gives to the skin its pale pink appearance.

However, few available and validated methods exist to study this notion with a global approach [1; 2; 3]. Actually, only very specific devices enable to have access to either parameter of the complexion radiance, giving incomplete information.

Our objective was to offer two models enabling to classify different profiles of complexion radiance according to whole parameters containing information of colour, transparency, state of fatigue, brightness, homogeneity but also skin texture.

We thus set up a method of sensory analysis by an expert judge in order to study the different parameters involved in the complexion radiance. Expert evaluated series of factors such as transparency, colour, brightness, microrelief... and studied the pertinence and impact of these factors on the notion of complexion radiance.

In parallel, the development of an images analysis method was carried out. Thanks to specific programs and algorithms of images analysis, this technique enables studying different areas of the face and giving quantitative information on different parameters (homogeneity, brightness, colour...).

Material and Methods:

Sensory evaluation:

Face complexion radiance was assessed blind by an expert previously trained to judge various representative parameters of complexion radiance. The study was carried out on more than 100 healthy volunteers, of all age. It enables to give a global appreciation of the complexion radiance but also to implement references profiles.

The assessment was based on scoring scales (1 to 10) for the following parameters:

- Skin grain: irregularity of the skin surface is correlated with a dull appearance of the skin.
- Transparency of the skin enables veinules to be seen. The thinner the skin, the more it allows light to pass, the sign of a healthy complexion.
- Reflection, of back-scattering, by the skin characterizes a radiant complexion. The higher the reflection of light by prominent zones of the face, the more radiant the complexion.
- Red colour is characteristic of microcirculation. A uniform complexion is characterized by a red colour component of low magnitude.
- Olive colour: this parameter represents a healthy complexion. The lower the olive colour, the more the skin is radiant.
- Beige colour is an individual characteristic since it depends on pigmentation. This parameter must change little or not at all in order to remain within identical conditions of evaluation before and after treatment.
- Pale pink colour characterizes a radiant complexion. The pinker the skin, the fresher it is perceived.
- Presence of wrinkles and lines: The more wrinkles and lines are present, the more there are skin surface irregularities creating shadows. Complexion seems younger, more luminous if lines and wrinkles are smoothed out.
- The state of eye fatigue and the presence rings: the complexion is more radiant when the skin is relaxed. Evaluating the appearance of the eyes aspect is a general assessment of the general fatigue of the skin.
- Overall appreciation of the complexion is the final judgment of the entire face using the reference scale described in the following sections.

These parameters were assessed on the cheekbones, forehead, chin and eyes.

Instrumental assessment:

Photos of volunteers were taken with the VISIA CR® (Canfield, USA) device, then, the following parameters of images were analyzed:

- Irregularities of the brightness: Very disparate and intensive brightness levels will tend to a dull complexion, in the contrary, a less bright skin but evenly will tend to a glowing complexion.
- Clarity of the complexion: clearer the face and more important the luminance.
- Intensity of the skin colour: the more the skin colour tends to a very pronounced colour (by example red), and the more saturation intensity will be important. In the contrary, a rosy complexion little coloured will have weak saturation intensity.
- Imperfections of the skin (spots, scars...) according to the contrast: the more the contrast will be important between the imperfection and the skin and the more the value of the parameter will be important.
- Circles: calculated in the eyes area by using the luminance parameter. Individual with pronounced circles will have a decrease of the luminance parameter in this area.
- Wrinkled aspect: assessed on the crow's feet area according to the contrast parameter which is representative of the depth of wrinkles according the intensity of this parameter.

Both approaches enabled us to define references profiles characteristic of different stages of the complexion radiance, then we analyzed profiles between smokers and non-smokers, and according to the age.

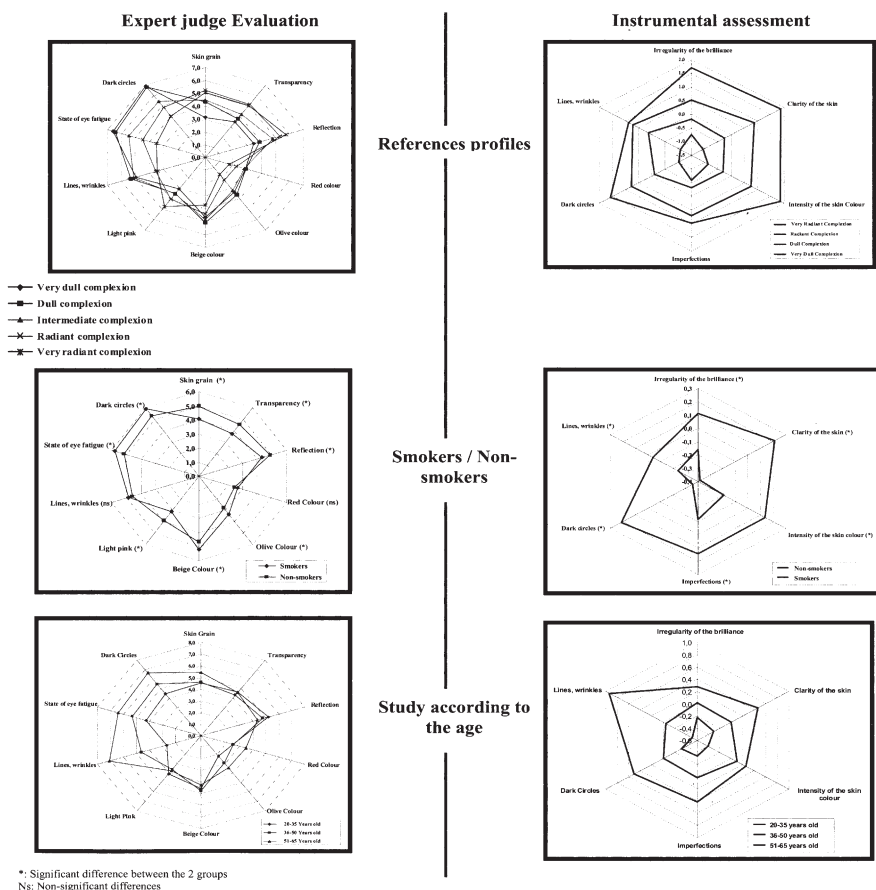
Results:

Figure 1: Graphics representative of profiles of the complexion radiance obtained in both approaches.

Conclusion:

The aim of this study was first of all to develop two complementary methods to assess skin complexion via a global approach thanks to instrumental and sensorial evaluation. By the way, the expert judge evaluation helps to get information on the visual perception of the skin complexion and gives a description of the skin state on different parameters while the image analysis method give information on parameters associated to the way light is returned by the skin.

By using two complementary ways of complexion radiance analysis, once sensorial and instrumental, according to multiple parameters, we defined different references profiles. Analysis of profiles of the complexion radiance in populations of smokers and non-smokers, showed a tendency to a dull complexion for smokers. Then, we showed that the complexion radiance was modified with age, in both evaluations (**Figure 1**).

These both approaches will be very useful and precious for pre-selecting *in-vivo* panelists, particularly for the development of natural active ingredients designed for the cosmetic industry and improving the complexion radiance.

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HOW THE POTENT SNAKE VENOM OF THE TEMPLE VIPER SERVED AS BIOMIMETIC DESIGN MODEL FOR A TREATMENT OF SKIN WRINKLES

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Abstract:

Waglerin-1 is the snake venom component of the temple viper that served as a biomimetic design model to develop muscle relaxing derivatives to treat facial skin expression wrinkles. Because the synthesis of a 22 amino acid peptide would be uneconomic for personal care applications, we screened for smaller analogs with similar activity, considering biomimetic principles. Waglerin contains several structural motives consisting of basic amino acids next to proline in the amino acid sequence. We assumed that these parts to be essential for the affinity to the biological target, the acetylcholine receptor. This observation was the starting point to design a couple of modified tripeptides with said structural motives in order to mimic the natural active molecule (waglerin) also by a shorter sequence. Several small peptides sequences had been synthesized and screened in an in-vitro nerve muscle cell co-culture assay. Indeed some of them considerably reduced the contraction frequency of the muscle cells. The best compounds were selected and further optimized to obtain adequate skin delivery properties. The lead candidate could be easily formulated into almost any skin care formulation and a double blinded clinical study with 45 volunteers vs. placebo showed remarkable benefits at 100 ppm use level. The study was an intra individual comparison with a before and after setup and performed at DermScan/Lyon. There were three test groups, each 15 subjects using either a placebo or a formulation with the Waglerin mimicking peptide H-beta-Ala-Pro-Dab-NHbenzyl or a formulation with a commercial hexapeptide. The products had been applied to the entire face twice daily. Wrinkle evaluation was done with a Primos 3D instrument by different surface parameters (Ra, Rz, Rt). The data were analyzed with a paired t-test. The placebo and the hexapeptide did not significantly improve ($p > 0.05$) any wrinkle parameter, while the Waglerin mimic showed a significant improvement at the forehead wrinkles. (Ra -21 %, Rz -20 %, Rt -15 %) (see Fig.2). The clinical results represented the last step in a successful biomimetic design project for expression line treatment product.

Introduction:

It is known that some of the most evident wrinkles in the face are so called expression lines resulting from repeated muscle contractions during facial expression. Relaxing contraction and tension of these muscles is a direct and fast treatment to reduce expression lines hence controlled intramuscular injections of highly diluted botulinum toxin has become popular to efficiently level out the expression lines. However important drawbacks are the application by a doctor's injection, the highly toxic potential and partial irreversibility, relatively expensive treatments and not well known is that the quality control of the toxin is dependent on extensive animal tests. The objective was to find an alternative ingredient with muscle relaxing properties but negligible toxic potential which is not dependent on injections but could be applied topically at home by the consumer. The venom of snakes is always a very complex mixture of several hundreds or thousands of components some of them with highly potent biochemical activity. One of the active components of the temple viper (*Tropidolaemus wagleri*) venom is a small polypeptide with 22 amino acids named waglerin-1(1). Waglerin has been shown to be a competitive antagonist of the muscle nicotinic receptor. The inhibition of this receptor prevents muscle activation and stops its contraction. Wagerin displaces alpha-Bungeratoxin from the nicotinic acetylcholine receptor (2) and the hypothesis for the structural design was that the active principle of the toxin is primarily based on the sequence motive of two basic amino acid (Arg or Lys) separated by a proline.

Waglerin-1 is a polypeptide with a chain length of 22 amino acids a molecular mass of 2522Da and an intramolecular disulphide bond between Cys9 and Cys13 (3)

H-Gly-Gly-Lys-Pro-Asp-Leu-Arg-Pro-Cys-His
Pro
Cys-Pro
His-Tyr-Ile-Pro-Arg-Pro-Lys-Pro-Arg-OH

Results:

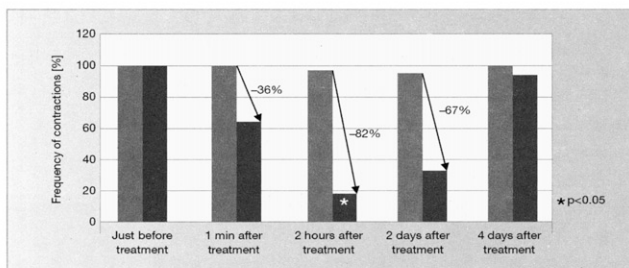
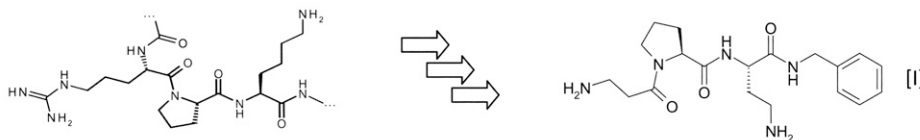


Fig1: For the in-vitro screening assay human muscle cells were co-cultured with rat neurons from dorsal root ganglia to obtain a neuromuscular junction model. Cultures were observed with an inverted microscope connected to a video recorder. The contraction frequency was measured before and after addition of the compounds. The blue columns show the contractions after addition of 0.5mM of the H-beta-Ala-Pro-Dab-NHbenzyl peptide derivative and the grey columns represent the untreated control

A rational active structure design was combined with an in-vitro screening program using about 20 different structures. The following modifications were necessary to improve the efficacy like reducing side chain length and basicity, formation of C-terminal amide and the benzyl group proved to be very useful for optimal dermal uptake arriving with the derivative [I] H-beta-Ala-Pro-Dab-NHbenzyl. The in-vitro screening was performed as described in Figure 1. In-vitro data not only measure the potency of action but also help to estimate the reaction time, duration of the effect and reversibility of the reaction.

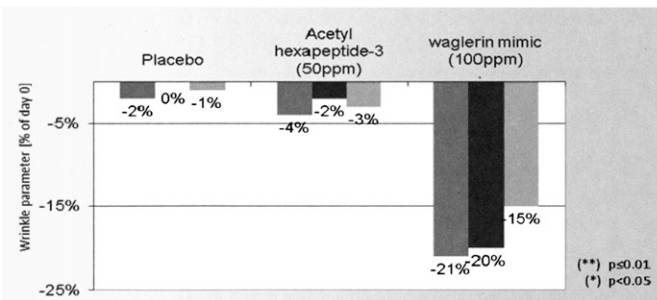


Fig2: The in-vivo study was a double blind and intra-individual study with 15 volunteers per group (female aged 41 – 60) with a twice daily application during 28days. Anti-wrinkle effect was evaluated using PRIMOS 3D at the crows feet and forehead. Shown here are the effects of 100ppm of the H-beta-Ala-Pro-Dab-NHbenzyl (waglerin mimic) applied in a cosmetic formulation at 100ppm and compared to commercially available acetyl hexapeptide-3 (50ppm)

Conclusion:

In conclusion this is a nice example how the fascinating natural source of a tailor made and highly potent snake toxin is taken as archetype to develop low molecular weight derivatives with adequate activity for safe, convenient and well tolerated skin applications.

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