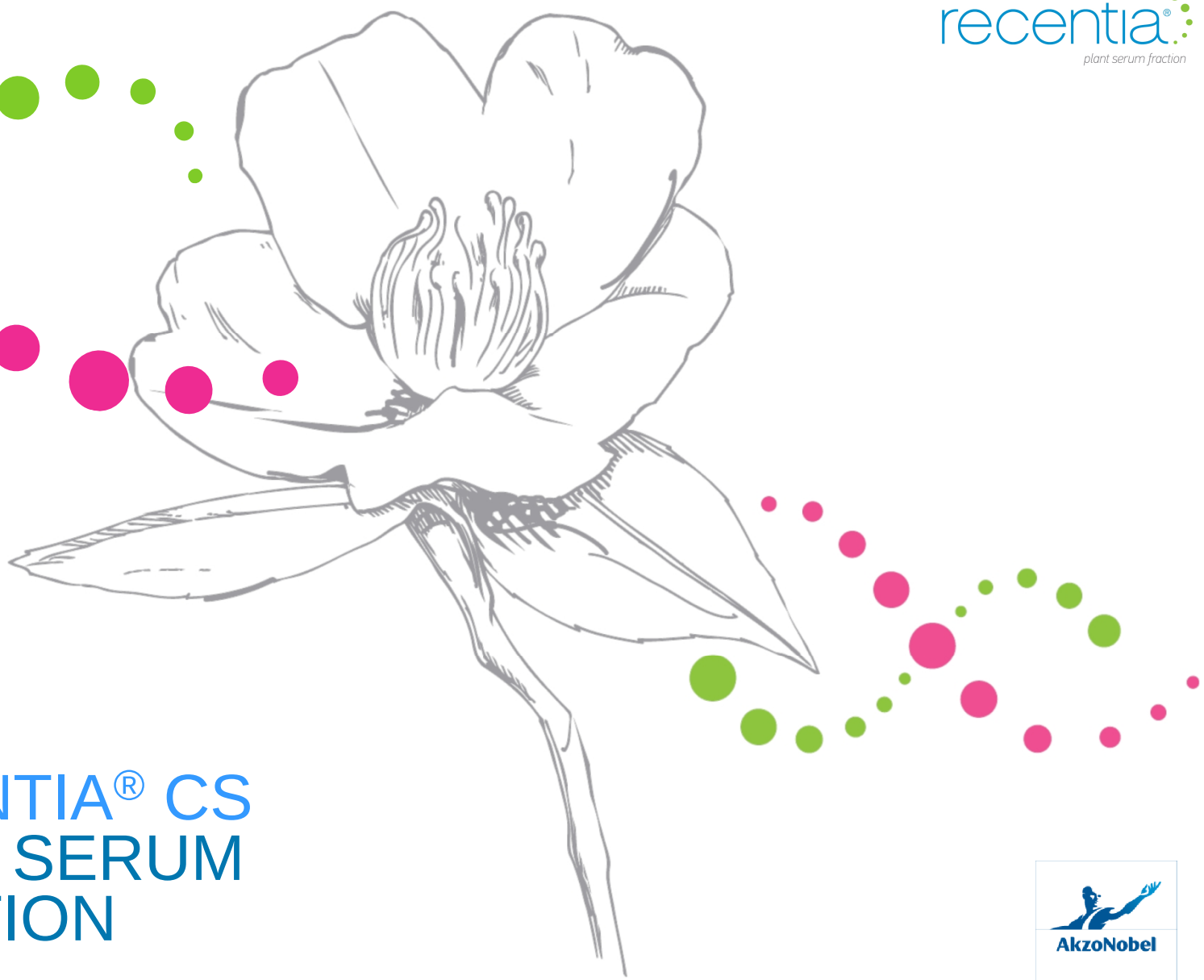
recentia®
plant serum fraction



RECENTIA® CS
PLANT SERUM FRACTION

AkzoNobel

DISCOVERING RECENTIA® CS

- Introducing RECENTIA® CS Plant Serum Fraction
- Understanding Skin Aging – Key Influences

  Drivers effecting anti-aging skin care
- Fresh Innovation

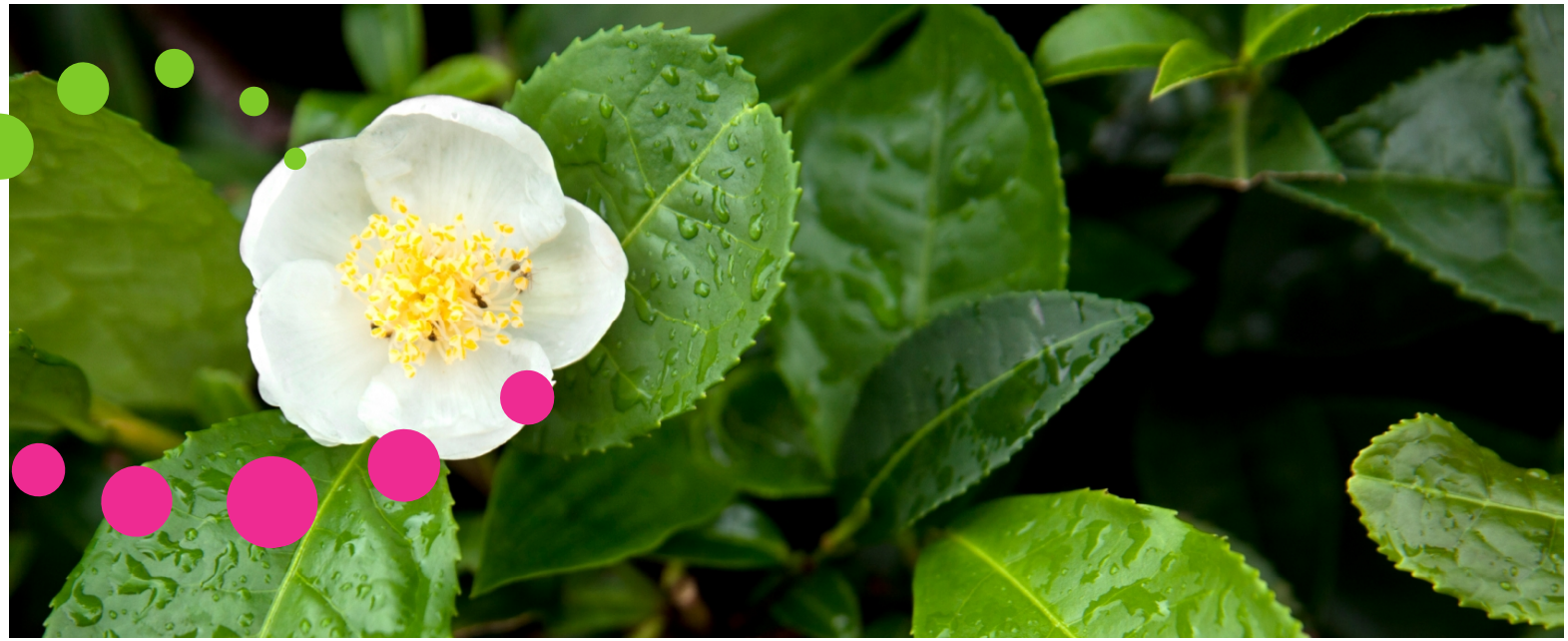
  Understanding the limitations of plant extracts vs. Zeta Fraction™ technology
- The Ingredient

  RECENTIA® CS plant serum fraction performance

  Efficacy testing
- Summary of the Results

INTRODUCING RECENTIA® CS

Rē- cěn- tiă (*Latin origin*): fresh, new, vigorous

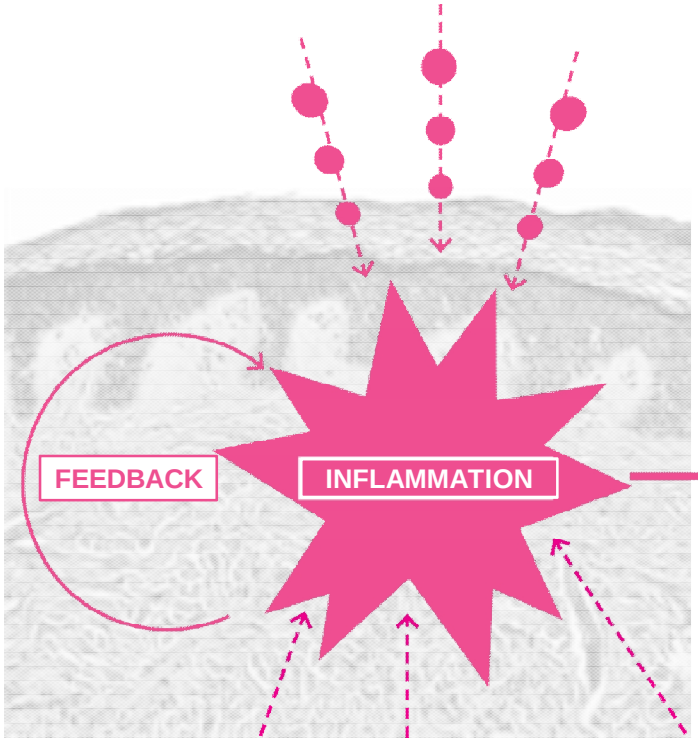


*Let RECENTIA® CS plant serum fraction helps you mitigate the signs of **skin aging- naturally**.*

UNDERSTANDING SKIN AGING

RECENTIA® CS plant serum fraction brings an entirely new level of efficacy and differentiation to skincare.

KEY MECHANISMS OF SKIN AGING



EXTERNAL FACTORS

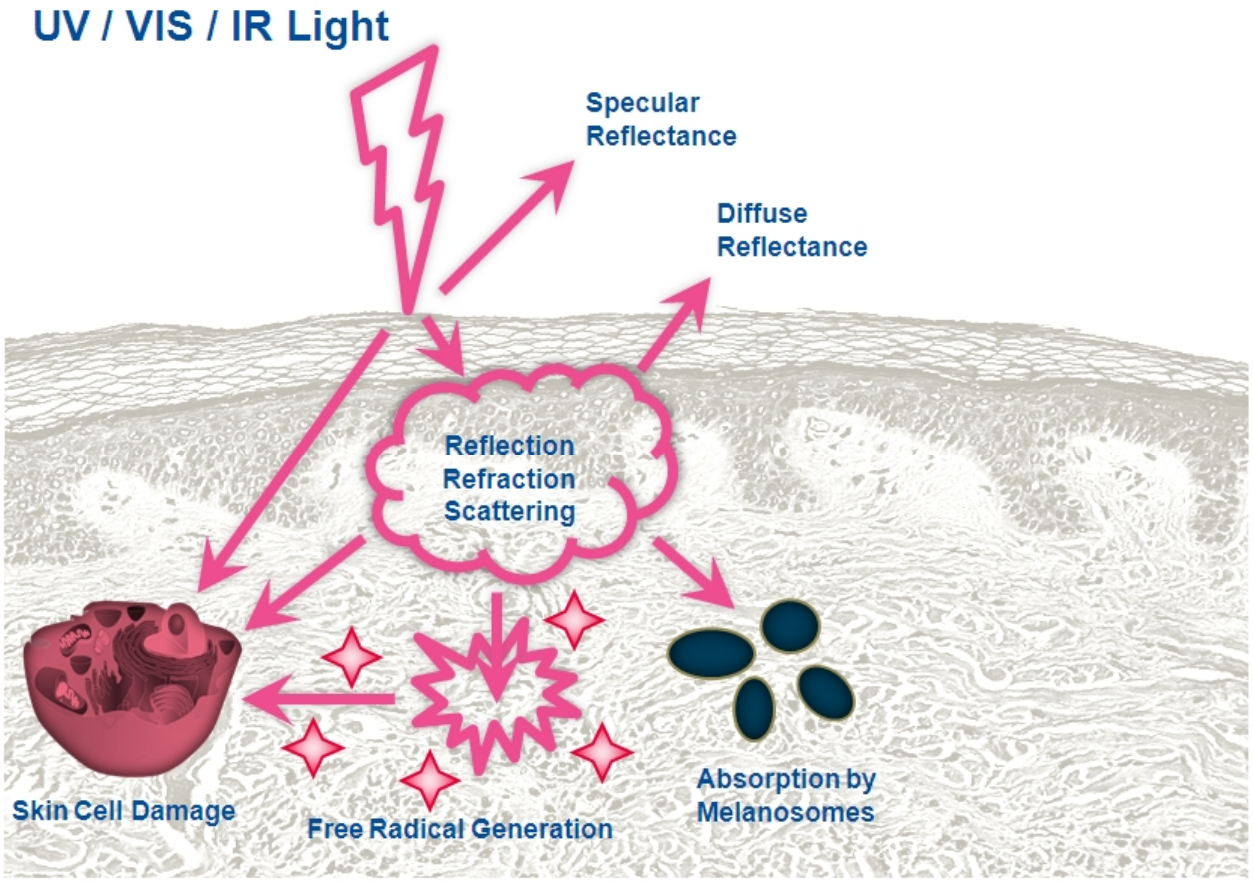
- **Photodamage: UV/VIS/IR light**
- Environmental pollutants
- Irritants

INTERNAL FACTORS

- Allergies
- Overactive immune response
- Age-related changes

Skin aging is the result of imperfect repair of cumulative damage caused by external and internal factors

SKIN TONE & DIFFUSE REFLECTANCE

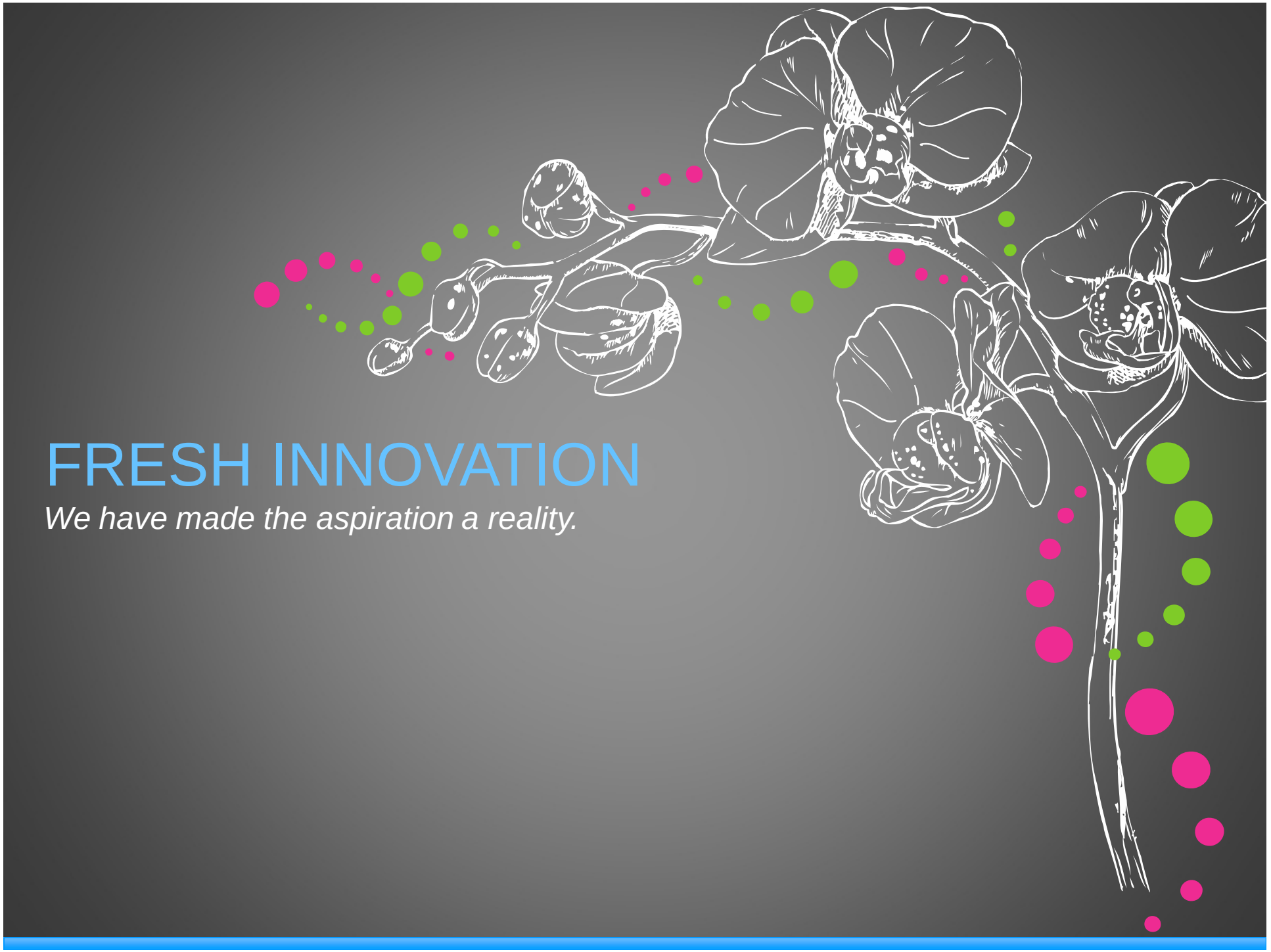


Diffuse reflectance of skin - a significant factor in photodamage

SKIN HEALTH DRIVERS

- Synergy between skin and sun care products inevitable in new product developments
- Antioxidant benefits that offer wide protection against free radicals are equally important in skin and sun formulations
- Understanding the level of UV/VIS free radical generation and the difference in diffuse reflectance on different skin types may significantly impact how we formulate.
- Multifunctional active ingredients are used to protect skin health from chronic inflammation

New generation skin care products will deliver instant gratification plus long-term benefits


FRESH INNOVATION
We have made the aspiration a reality.

recentia®
plant serum fraction

EXTRACTION & SEPARATION METHODS

BASIC APPROACH

- Drying of Plants
- Solvent or Supercritical Extraction
- Separation based on:
  - Crystallization
  - Evaporation
  - Distillation
  - Membrane filtration
  - Chromatography
  - Electrophoresis

LIMITATIONS AND CONCERNS

- Contamination by Residual Chemicals
- Deterioration of Stability, Color & Odor
- Increased Toxicity & Allergenicity
- Decreased Activities & Bioavailability
- Inability to Produce Valuable Byproducts
- Decreased Yield
- Poor Reproducibility
- High Scale Up Risk
- Low Effectiveness
- Not Sustainable Process

recentia®
plant serum fraction

ZETA FRACTION™ TECHNOLOGY

NOVEL APPROACH

- FRESH START
  - Collection of **Living** Plants
- NATURAL ENVIRONMENT
  - Mechanical Separation
  - Preserve Inherent Osmotic Pressure of Intracellular Content
  - Use Intracellular Content as Media for Separation
- SEPARATION BASED ON
  - Localization in Cell
  - Affinity to Biomembranes
  - Colloidal properties

POSITIVE RESULTS

+ No Residual Solvents/Chemicals
+ Minimum Deterioration of Stability, Color & Odor
+ Minimal Toxicity & Allergenicity
+ High Level of Activities & Bioavailability
+ Preserves Whole Spectrum of Existing Biological Activities
+ Increased Yield & Reproducibility
+ Decreased Scale Up Risk
+ High Efficiency
+ Fully Sustainable Process

THE INGREDIENT

Derived from a living plant source, RECENTIA® CS plant serum fraction offers unparalleled benefits.

RECENTIA® CS PLANT SERUM FRACTION

- The first Zeta Fraction™ ingredient (US 7,473,435 and US 8,043,635) launched to the global personal care market
- Obtained from living *Camellia sinensis* (Tea plant) - one of the most important plants in the history of mankind
- Uses patented Zeta Fraction™ technology to deliver fresh *Camellia sinensis* bioactive ingredient

RECENTIA® CS PLANT SERUM FRACTION

TYPICAL PROPERTIES

INCI: *Camellia sinensis Flower/Leaf/Stem Juice*

Appearance	Yellow Liquid
Odor	Characteristic
Solubility in Water	Soluble
Dry matter (% weight)	6.5 - 9.0
Refractive index (nD)	1.34 - 1.35
Density (g/cm^3)	1.03 - 1.06
pH	3.7 - 4.4
UV_{max} (nm)	267 – 269
CAS RN	1196791-49-7

RECENTIA® CS PLANT SERUM FRACTION:
SAFETY/TOX PROFILE

TOXICOLOGICAL ENDPOINT	MODEL	RESULTS
Skin Irritation	MatTek EpiDerm™	Non-irritant*: No effect on cell viability (~98- 103% of control); No significant increase in IL-1a
Ocular Irritation	MatTek EpiOcular™	Non-irritant*: No effect on cell viability (~93-98% of control); No significant increase in IL-1a
Genotoxicity	*Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537; E. coli strain WP2uvrA	Nonmutagenic: no significant effect on mean number of revertants vs. negative control
Acute *Daphnia magna* Toxicity	*Daphnia magna*	No significant effect: EC_{50} >1000 mg/L; NOEC 1000 mg/L
Algal Growth Inhibition	*Desmodesmus subspicatus*	No significant effect: EC_{50} >1000 mg/L; NOEC 250-1000 mg/L

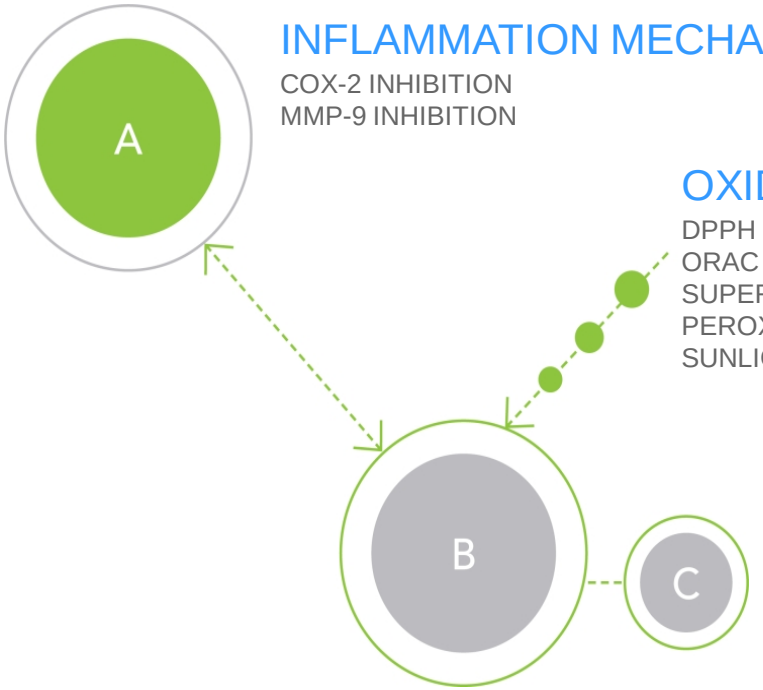
** tested at 5%*

recentia®
plant serum fraction

OPPORTUNITIES TO MITIGATE SKIN AGING

- **Minimize Formation of Reactive Oxygen Species & Free Radicals**
- **Prevent Oxidative Damage**
- Up-Regulation of Intracellular Anti-Oxidants
- **Inhibition of COX-2**
- Minimize Formation of Inflammatory Messengers (e.g. eicosanoids, cytokines & chemokines)
- Increase Production & Release of TGF-β
- Up-Regulation of Anti-Inflammatory Molecules (e.g. IL-1 receptor antagonist & TNF receptor)
- De-Sensitization of Receptors
- **Inhibition of Proteases (e.g. elastase, MMPs, trypsin)**
- Inhibition of Nitric Oxide Release by Macrophages
- Increase the Apoptosis of Pro-Inflammatory Cells
- Increase Survival of Cells due to Interactions with Extracellular Matrix

IMPACT ON SKIN AGING
TESTING PERFORMED



INFLAMMATION MECHANISMS
COX-2 INHIBITION
MMP-9 INHIBITION

OXIDATIVE/ FREE RADICAL DAMAGE
DPPH
ORAC
SUPEROXIDE ASSAY
PEROXIDE PRODUCTION ASSAY
SUNLIGHT GENERATED FREE RADICALS

INGREDIENT INSTABILITY
UVAPF PHOTOSTABILITY

PREVENTION OF INFLAMMATION
CYCLOOXYGENASE-2 INHIBITION ASSAY

THE RELEVANCE
Measures ability to inhibit synthesis of key inflammation messenger substances: prostaglandins.

THE ASSAY
An enzyme-linked immunosorbent assay allows endpoint colorimetric determination of the amount of prostaglandins in a sample and, consequently, activity of COX-2. Inhibitors slow or stop the prostaglandin synthesis by COX-2, producing lower readings.

THE RESULT
RECENTIA® CS plant serum fraction* IC_{50} ≤ 0.15% v/v in reaction volume.

**liquid material as supplied (~8% dry matter)*

Inhibits production of inflammatory messengers

PREVENTION OF INFLAMMATION

MATRIX METALLOPEPTIDASE-9 INHIBITION

THE RELEVANCE

Measures the ability to inhibit a proteolytic enzyme critical to aging process. This enzyme can degrade extracellular matrix during inflammation.

THE ASSAY

MMP-9 is captured by immobilized antibodies. Any active MMP-9 activates a pro-detection enzyme, enabling it to cleave a normally colorless substrate into colored form. Inhibitors slow or stop the process, producing lower rates and endpoints of color development.

THE RESULT

RECENTIA® CS plant serum fraction* estimated $IC_{50} \leq 0.03\%$ v/v in reaction volume.

** liquid material as supplied (~8% dry matter)*

PREVENTION OF OXIDATIVE DAMAGE:
DPPH ASSAY

THE RELEVANCE
Measures free radical quenching ability of test article

THE ASSAY
Using DPPH (2,2-diphenyl-1-picrylhydrazyl) as the stable free radical compound, the assay is a kinetic colorimetric measurement . When DPPH is quenched the color changes from dark purple to pale yellow.

THE RESULT
11 units weight RECENTIA® CS plant serum fraction*
quench 1 unit dry weight DPPH

** liquid material as supplied (~8% dry matter)*

PREVENTION OF OXIDATIVE DAMAGE:
ORAC ASSAY

THE RELEVANCE
Measures ability of test article to quench reactive oxygen species

THE ASSAY
Oxygen Radical Absorbance Capacity, a kinetic fluorometric assay, determines the activity of test article compared to known antioxidant, (R)-Trolox methyl ether by measuring its ability to protect the fluorophore.

THE RESULT
10 units weight RECENTIA® CS plant serum fraction* have effect equal to 1 unit dry weight (R)-Trolox methyl ether

** liquid material as supplied (~8% dry matter)*

PREVENTION OF OXIDATIVE DAMAGE:
SUPEROXIDE SCAVENGING ASSAY

THE RELEVANCE
Measures the ability of test article to scavenge ubiquitous physiologically important reactive oxygen species

THE ASSAY
An enzymatic reaction generates superoxide anions which reduce cytochrome *c* to differently colored form

Superoxide scavengers slow or stop this process, producing lower rates and amounts of color change

A kinetic colorimetric assay allows determination of efficacy of superoxide scavengers expressed as ICR_{50} – a concentration which prevents 50% of cytochrome *c* from being reduced

THE RESULT
RECENTIA® CS plant serum fraction* $ICR_{50} \leq 0.14\%$ v/v in reaction volume

** liquid material as supplied (~8% dry matter)*

PREVENTION OF OXIDATIVE DAMAGE

PEROXIDE PRODUCTION ASSAY

THE RELEVANCE

Measures the ability of test article to decrease levels of hydrogen peroxide in skin cells

THE ASSAY

Human epidermal keratinocytes perfused with peroxide-sensitive fluorescent molecular probe (DCFDA*) were treated with different concentrations of RECENTIA® CS plant serum fraction for 5 minutes, both with and without exposure to 60 µM hydrogen peroxide.

THE RESULT

RECENTIA® CS plant serum fraction** at 2% v/v concentration significantly (by ~92%) reduces peroxide-induced fluorescence in peroxide-treated cells.

** 2',7'-dichlorofluorescin diacetate (DCFDA)*
*** liquid material as supplied (~8% dry matter)*

PREVENTION OF OXIDATIVE DAMAGE

SUNLIGHT-GENERATED FREE RADICAL ASSAY

THE RELEVANCE

Scavenging and quenching free radicals produced by sunlight irradiation

THE ASSAY

The substrate containing phospholipid liposomes that also mimics color and diffuse reflectance of light skin is used for generation of free radicals by solar simulator irradiation, with added molecular probes used to measure free radical activity. Adding antioxidants and free radical scavengers lowers the induced probe fluorescence

THE RESULT

0.35% v/v of RECENTIA® CS plant serum fraction* in reaction volume inhibits fluorescence induced by 10 MED irradiation by ~50% for DCFDA and by ~45% for SOSGR

* liquid material as supplied (~8% dry matter)

Protects biological molecules from sunlight-generated ROS

PREVENTION OF OXIDATIVE DAMAGE

UVAPF PHOTOSTABILITY ASSAY

THE RELEVANCE

Measures the ability to photo-stabilize active product ingredients susceptible to UV radiation

THE ASSAY

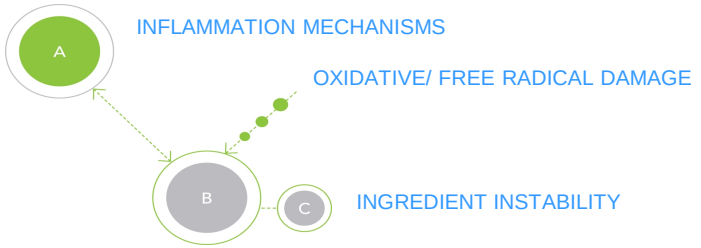
Determine the sun protection factor of sunscreen formulation on skin equivalent before and after simulated solar radiation. Addition of ingredients that protect actives from photodamage results in applied formulation retaining higher protective values after irradiation.

THE RESULT

1% w/w RECENTIA® CS plant serum fraction* in test formulation results in 25% less UVAPF loss as a result of 25 MED irradiation

** liquid material as supplied (~8% dry matter)*

recentia®
plant serum fraction



MULTIFUNCTIONAL PERFORMANCE

TARGET BENEFIT TYPE	ASSAY	RESULTS	WHAT DOES IT MEAN?
A	COX-2 Inhibition CYCLOOXYGENASE 2	IC_{50} < 0.15% v/v in reaction volume	Anti-inflammatory properties at low concentrations
A	MMP-9 MATRIX METALLOPEPTIDASE 9	estimated IC_{50} ≤ 0.03% v/v in reaction volume	Anti-inflammatory properties at low concentrations
B	DPPH	11 units CS quench 1 unit dry weight DPPH	Very potent free radical quencher
B	ORAC	10 units CS have activity equal to 1 unit dry weight (R)-Trolox methyl ether	Very potent antioxidant
B	Peroxide production assay	2% v/v decreases cell peroxide content by ~70% (endogenous H_2O_2 only) or >90% (endogenous +60 µM exogenous)	Potent antioxidant (cell culture based assay)
B	Superoxide assay	ICR_{50} ≤ 0.14% v/v in reaction volume	Potent superoxide scavenger
B	Sunlight generated free-radicals	0.35% v/v in reaction volume after 10 MED irradiation inhibits ~50% DCFDA and ~45% SOSGR fluorescence	Very potent, sunlight-generated free-radical quencher
C	UVAPF	1% w/w in sunscreen formulation reduces UVA PF loss by 25% after 25 MED	Ability to photostabilize ingredients

RECENTIA® CS PLANT SERUM FRACTION

DELIVERING NATURE'S POTENTIAL

- Protect skin against age-associated damage
- Quench free radicals and reactive oxygen species that trigger inflammatory responses
- Slow production of inflammation messengers
- Fight against tissue-damaging enzymes
- Protect other formulation ingredients from photodamage
- Developed from truly green technology

RECENTIA® CS plant serum fractions bring an entirely new level of efficacy and differentiation to anti-aging skin care.

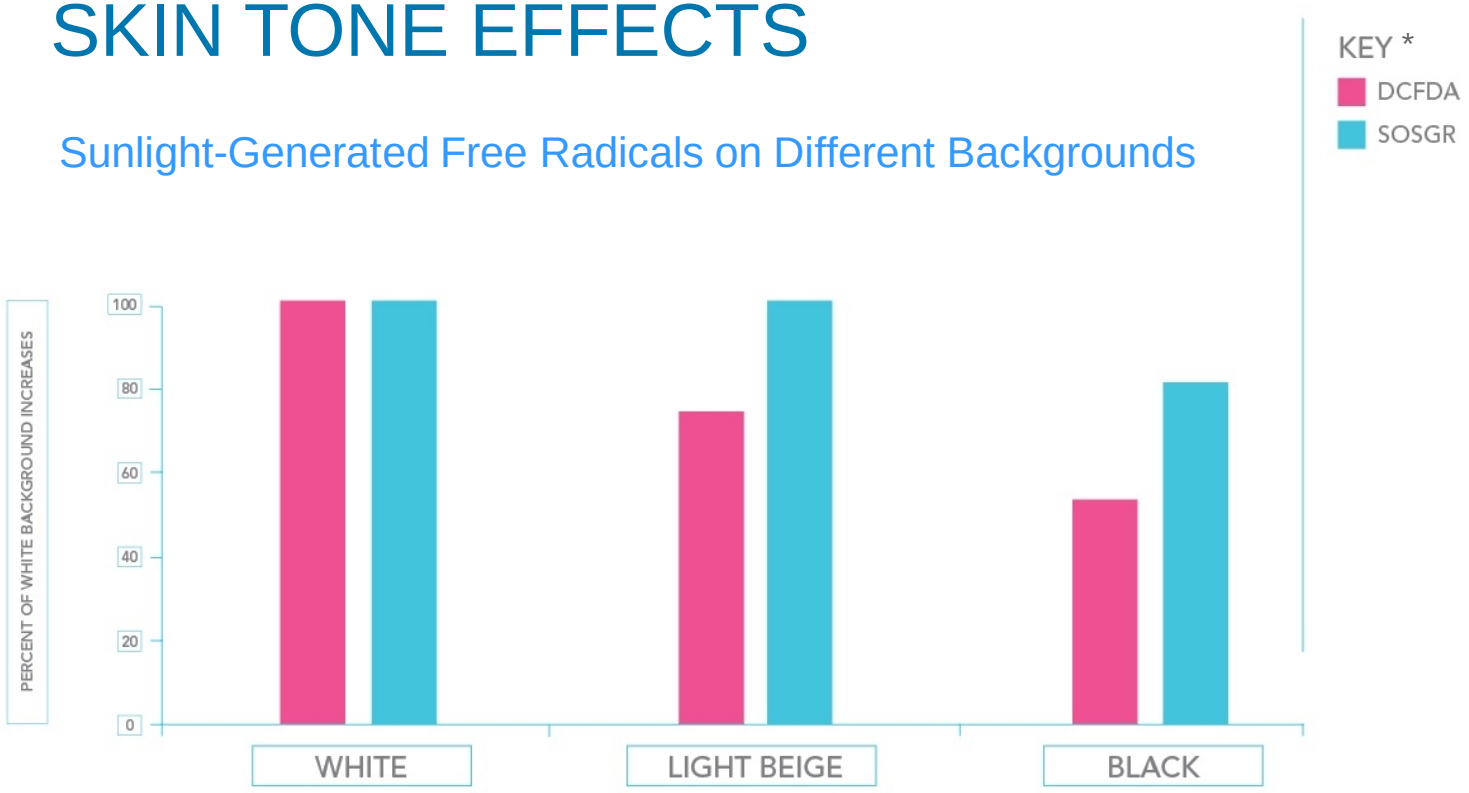
THANK YOU

AkzoNobel

BACK OF DECK

SKIN TONE EFFECTS

Sunlight-Generated Free Radicals on Different Backgrounds



- Black background color shows less increase in fluorescence, which corresponds to less free radical production
- These data provide the plausible explanation for some sunscreens being more photostable on dark backgrounds, such as those explicitly selected for minimizing diffuse reflection

* SOSGR: Singlet Oxygen Sensor Green Reagent
DCFDA: 2',7'-dichlorofluorescin diacetate

recentia®
plant serum fraction

WHAT'S BEING OVERLOOKED?

- Diffuse reflectance of various color types of human skin in UVA/VIS area is markedly different

DIFFUSE REFLECTANCE RATIOS AMONG HUMAN SKIN COLOR TYPES		
SKIN TYPE I / SKIN TYPE V	SKIN TYPE II / SKIN TYPE IV	SKIN TYPE II / SKIN TYPE III
3.1	2.3	1.4

- The diffuse reflectance of Caucasian skin in UV-VIS area is about 3 times higher compared with darker skin
- Higher diffuse reflectance of lighter skin color types, especially in UVA-VIS area, increases the probability for more photons to be reflected – not absorbed – and to react with components of the skin
- Newly developed *in vitro* test methodology addresses this issue (US 2011/0300572 A1)

MOVING FORWARD

RECENTIA® CS plant serum fraction plays a role in each of the key megatrends outlined below.

DEMOGRAPHICS

GEOPOLITICS

CONSUMER TRENDS

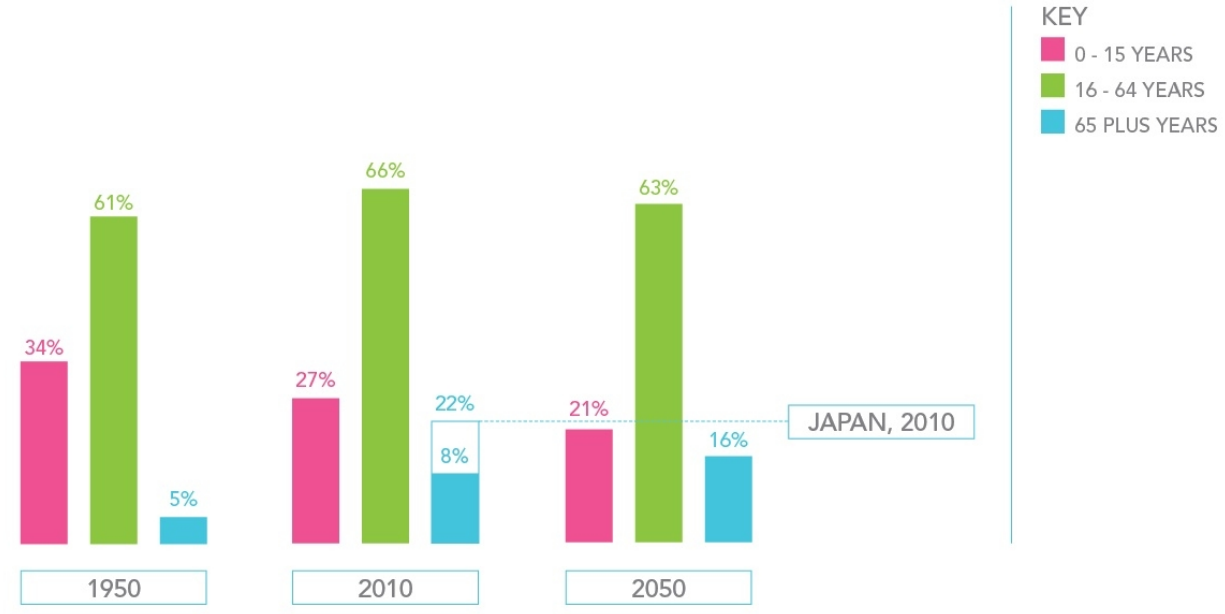
ENVIRONMENT

TECHNOLOGY

NEW MARKET OPPORTUNITIES
SPANNING GENERATIONS

World Population Distribution by Age

Source: United Nations, World Population Prospects: The 2012 Revision; BCG Analysis



Both young and old offer new market opportunities.

THE NATURAL PROGRESSION

Imagine the ability to access the full spectrum of bioactives in the world's most potent plant species.

recentia®
plant serum fraction

FORMULATE BETTER

- Something entirely new
- Truly natural
- Totally sustainable
- Unmatched safety profile
- Pleasing aesthetics
- Product of activity-driven separation
- Better formulations

recentia®
plant serum fraction

POTENT ANTI-AGING PERFORMANCE

- Multiple activities that work together to protect skin against damage associated with aging
- Quenches damaging free radicals and reactive oxygen species that also trigger inflammatory response
- Slows production of inflammation messengers
- Protects against tissue-damaging enzymes
- As potent antioxidant can protect other formulation ingredients from photodamage
- Product of a truly green technology
- Comes from a plant recognized worldwide for its health benefits