EVALUATION OF THE PRODUCT EFFICACY ON HYDRATION, ELASTICITY, pH, MELANIN LEVEL and WRINKE REDUCTION ON THE BASIS OF INSTRUMENTAL EVALUATION

1. GENERAL INFORMATION

STUDY SPONSOR	Skin Ingredients Pty, Cape Town, South Africa
STUDY CODE	C017/005
NAME AND ADDRESS OF THE ORGANIZATION IN CHARGE OF THE ASSESSMENT	Luamed, Tanja Židan s.p. Lukovica, Slovenia
TEST PRODUCT	Sk.in Gloss
AUTHORIZED BY	Tanja Židan
ADDITIONAL INVESTIGATIONS INVOLVED	Katja Urek
REPORT DATE	23.11.2019

2. PURPOSE AND OBJECTIVE OF THE TEST

The object of this test was to define the direct influence of the tested product on the level of skin hydration, elasticity, pH, melanin content and wrinkle reduction and to confirm the declared properties and efficacy of the product on the basis of instrumental methods and use test (consumer evaluation).

DESCRIPTION OF THE PRODUCT

INTENDED USE	Face care product
APPEARANCE	Cream
COLOR	White
FRAGRANCE	Characteristic
INSTRUCTIONS FOR USE	use every 2nd morning for 2 weeks then every morning. all volunteers continue to use their normal basic skincare regime such as cleanser, moisturiser and SPF and to slot the serums in.
PRODUCT CLAIMS AS DECLARED BY CUSTOMER	sk.in gloss is effective in improving hydration, reducing pigmentation, increasing collagen production, and improving fine lines and wrinkles. sk.in gloss can be used by all skin types.

INCI LIST (QUALITATIVE COMPOSITION) AS DECLARED BY CUSTOMER

The qualitative composition was delivered to the laboratory by the Sponsor, before the start of the study.

3. TEST SCHEDULE

STARTING DATE	27.9.2019
FINISHING DATE	22.11.2019

4. METHODOLOGY

PROTOCOL SUMMARY

Instrumental test using: Callegari 1930 Company – Soft Plus device (hydration probe, pH, elasticity, melanin probe, micro camera)

The aim of the test was to determine the direct influence of the tested product on skin hydration, elasticity, pH, melanin content and wrinkle reduction. The test was conducted with a special measuring device manufactured by Callegari 1930 Company – Soft Plus.

The instrumental measurements were performed on facial skin. Tested and control zones were indicated on the left part of the face. The application zone was an area of approximately 283 cm^2. The measurements were carried out for each zone in all tested and control places immediately prior to the application of the tested product. Subjects were told not to rinse off and to continue with their basic skincare regime one until the end of the study (8 weeks).

The measurement of the hydration effect was performed one, three and five hours after the product application. The arithmetic mean of the measurements of each of the 10 subjects is considered as each final result.
The measurement of the elasticity, melanin content and pH were performed three times, at the beginning, after 4 weeks and at the end of the study (8 weeks). The arithmetic mean of the measurements of each of the 10 subjects is considered as each final result.
The measurement of depth and degree of wrinkles was performed at the beginning and at the end of the study (8 weeks). Before and after fotos were recorded to evaluate the changes of the 10 subjects.

All measurements were carried out in a room with a temperature of 20 ± 3°C and a relative humidity of 50±10%. Directly before testing, the skin of the volunteers was gently wiped with warm water.

HYDRATION MEASUREMENT

The skin is divided into three layers: the epidermis, the dermis and the subcutaneous tissue.

A very thin hydro-lipid layer is found above the epidermis. This is basically made up of the secretions of the sebaceous glands and it maintains the skin's firmness and prevents the excessive loss of transcutaneous water and the entry of harmful substances.

The epidermis itself is composed of five different layers. In the bottom layer, the stratum basal, the cells divide and push already formed cells into the higher layers. As the cells move into the higher layers, they flatten and eventually die. The top layer of the epidermis, the stratum corneum, is made up of dead, keratinized cells that shed about every two weeks.

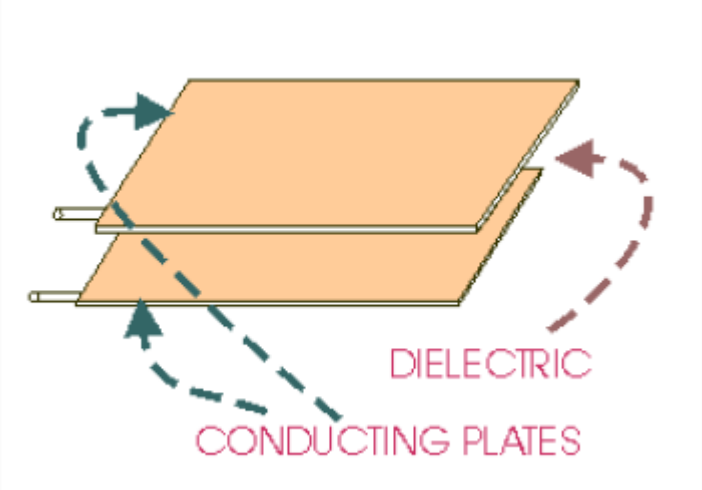
While the amount of water in the inner layers of the skin is relatively constant and is in equilibrium with the other organs of the body (ca. 60–70%), the moisture in the stratum corneum depends on different factors:

- the rate at which the water in the dermis reaches the stratum corneum
- the rate at which the water is eliminated by evaporation (TEWL)
- the ability of the stratum corneum to retain water.

When speaking about the skin's moisture, we refer to the moisture contents of the stratum corneum.

Soft Plus measures the hydration of the stratum corneum through the scientifically acknowledged capacitive method, which measures the amount of electric current passing through a capacitor.

In its simplest form, a capacitor consists of two conducting plates (tracks) that are separated by an insulating material called the dielectric.



In the capacitor constituted by probe and skin, one conducting plate is the probe surface and the other is represented by the deeper (well-hydrated) layer of the skin.

The horny layer, constituted by dead keratinized cells dispersed in a lipid medium, represents an excellent barrier to the passage of both chemical substances and electric current and it can, therefore, be considered a dielectric medium (the dielectric constant of the anhydrous horny layer is usually lower than five). The water dielectric constant is much higher (81), thus, if water is contained in the stratum corneum, the horny layer dramatically changes its dielectric properties.

In measuring the amount of current that flows through the stratum corneum, it is possible to evaluate the dielectric constant of the hydrated stratum corneum and to calculate its moisture content.

ELASTICITY MEASUREMENT

Every day our skin undergoes various mechanical stresses that can lead to its deformation. Different types of forces can deform the skin: lifting, tension, torsion, pressure and compression. When these forces are halted, the skin returns to its original position because of its elasticity. The measurement of skin elasticity is highly complicated for two reasons:

- the forces involved are very small and so they are measured with difficulty,
- collagen and elastin fibres stabilize the skin and prevent it from being torn in every direction of the space.

For these reasons, all of the methods described in the literature for the measurement of skin elasticity provide an indication of the condition of the skin.

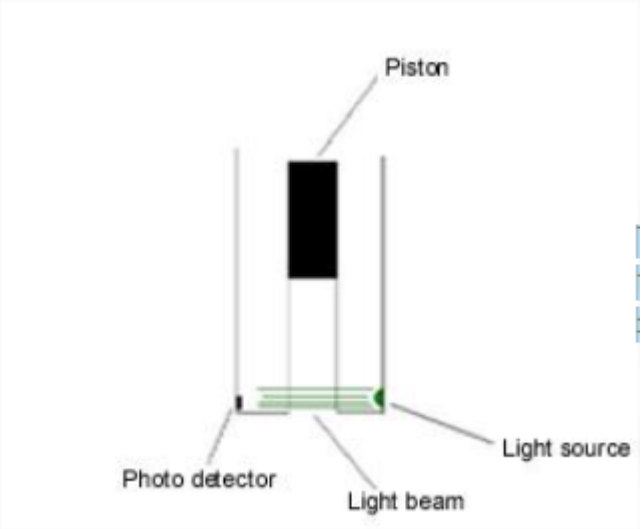
The measurement principle employed in the elasticity probe is based on the suction method.

From a technical point of view, pressing and releasing the piston creates a vacuum inside the probe (the probe is endowed with a small pump, thus, assuring the application of the same and precise vacuum for each measurement).

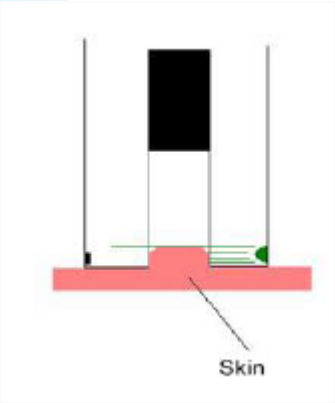
The skin reacts to this stimulus, rising into the chamber of the probe.

The cutaneous deformation is quantified through a sensor developed specifically for this purpose.

The sensor is made up of a light source that produces a transverse light beam on the inside of the probe. The more the skin is deformed on the inside of the probe, the more intense the decrease in the light beam. The decrease of the signal is correlated with the elasticity of the skin.



When the skin does not penetrate the inside of the probe, the quantity of light detected by the photodiode is considered to be maximum.



When the skin is not able to oppose resistance to the mechanical stress caused by the suction, it will penetrate the internal part of the probe.

The amount of skin that penetrates is measured by the reduction in the light that reaches the photodiode.

pH MEASUREMENT

The pH of a solution is defined as –log10 H3O+.

Pure water is partially dissociated in accordance with this equilibrium:
2 H2O ↔ H3O+ + OH-
The concentration of ions H3O+ is identical to those of ions OH- and the pH = 7.

When acids, bases or salts are dissolved in water, the concentration of ions bearing an opposite charge differs and the pH is ≠ 7.

Skin pH is usually slightly acid, which is mainly due to lactic acid and various amino acids from sweat, free fatty acids from sebum and amino acids and pyrrolidone carboxylic acid from the cornification process of the skin.

The measurement of skin surface pH is used in clinical research to evaluate shifts in pH following external exposures and to evaluate the state of diseased skin with acute or chronic changes.

It is possible to use different methods for the measurement of pH:

- the colourimetric method, which uses a particular chemical indicator (a shift in pH is paralleled with a visible shift in colour)
- the potentiometer pH measurement, which is the most precise and easy method used today.

The measurement of pH employed for the Soft Plus is based on the potentiometer principle.

In this procedure, electrical impulses are measured using an electrode and the difference in potential is measured with a potentiometer. The sensor is a glass electrode with selective hydrogen-ions sensitivity and has the highest sensitivity and reliability. This is formed by a glass bulb in which the active electrode (which is in contact with the skin) and the reference electrode (plunged into a solution of KCl) are combined. The small amount of water lying between the electrode and the skin surface is sufficient to dissolve the ions that are present on the skin surface (lactate, etc.) and to create adequate measuring conditions.

When the pH probe is in contact with a solution containing ions H3O+, a flux of electrons moves between the active electrode and the reference electrode: the potentiometer measures the difference in potential, which is directly proportional to the pH of the test solution.

The reactions that occur at the electrodes are shown below:
H3O+ + e- ↔ ½ H2 + H2O active electrode
Cl- ↔ ½ Cl2 + e- reference electrode.

A planar electrode was developed for the specific requirements of skin pH measurements. The planar electrode has several advantages. The electrode membrane is flat and the

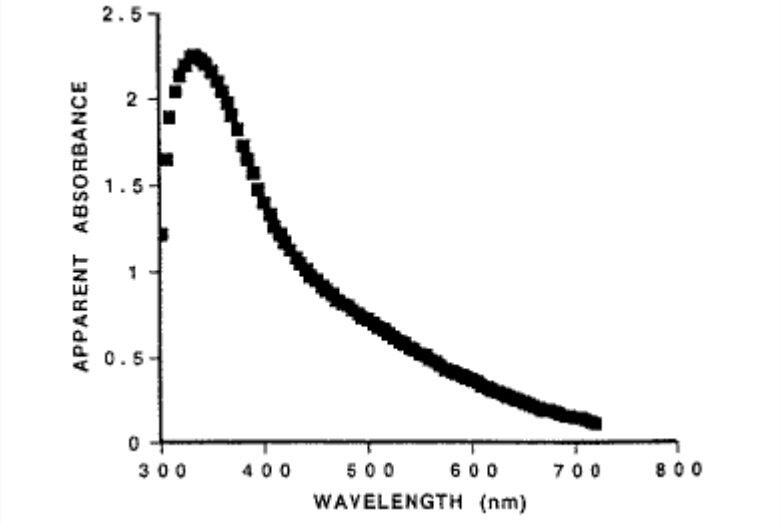
contact between the electrode and the skin surface covers a relatively large area. Use of an electrode is non-invasive and the electric current is small and constant and causes no damage.

MELANIN MEASUREMENT

The melanin technology employed is based on the absorption/reflecting principle at two defined wavelengths (875 nm and 660 nm).

Melanin is the most important pigment in determining skin colour in humans. Other substances that mainly contribute to the colour of the skin are oxy- and deoxyhaemoglobin and carotenoids.

While oxy- and deoxyhaemoglobin and carotenoids have a peculiar absorbing spectrum in the visible range, melanin polymer is neither well characterized nor unique and it does not have a typical band in the visible range. As expected, it has a maximum of absorbance in the UV portion of the spectrum (according to its biological function) and its absorptive capacity decreases with increasing wavelength (see image below).



The sensor of the probe is constituted by two kinds of LEDs emitting in the visible (660 nm) and IR (875 nm) spectrum and by a photodetector. The chosen wavelengths correspond to the different absorption rates of the pigments of the skin.

Once the probe has been applied to the skin surface, the radiation emitted by the light sources is partially absorbed and partially reflected by the skin.

The radiation reflected by the skin reaches the photodetector that measures the amount of energy both at 660 nm and 880 nm. The effect of melanin is observed as the decrease of reflectance in all bands.

DESCRIPTION OF VOLUNTEERS

INCLUSION CRITERIA	**GENERAL** Skin without irritation and changes requiring pharmacological treatment	**SPECIFIC** Age: 35-65 Skin type: Normal skin Amount: 10
EXCLUSION CRITERIA	- Skin diseases or any other medical condition requiring systemic medical treatment or which may interfere with the objectives of the study.	

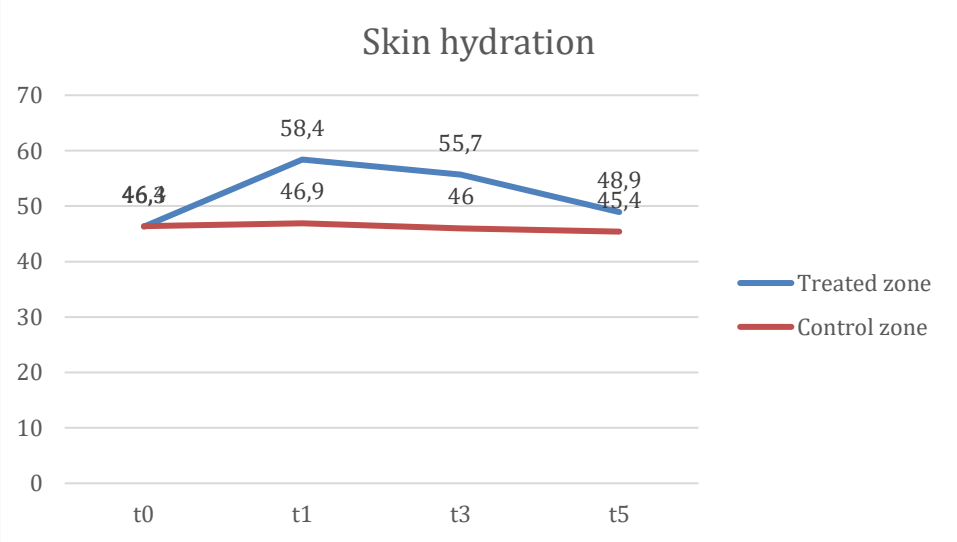
5. RESULTS

PRESENTATION OF RESULTS-HYDRATION MESUREMENT

Table 1: The results of skin hydration measurement before application (t0) and 1, 3 and 5 hours after the product application in arbitrary units. Each result is the average of 5 individual measurements.

	Treated zone				Control zone			
SUBJECT CODE	t0	t1	t3	t5	t0	t1	t3	t5
001	34	42	41	36	35	33	34	33
002	54	70	65	57	57	57	59	55
003	54	67	65	57	55	53	51	54
004	35	43	42	37	32	33	35	36
005	54	71	65	57	58	55	53	53
006	48	60	58	50	46	50	51	48
007	22	28	26	23	21	22	21	19
008	60	74	72	64	59	60	59	58
009	42	56	51	45	42	47	42	40
010	60	73	72	63	59	59	55	58
MEAN	46,3	58,4	55,7	48,9	46,4	46,9	46	45,4
MIN	22	28	26	23	21	22	21	19
MAX	60	74	72	64	59	60	59	58
MEDIAN	51	63,5	61,5	53,5	50,5	51,5	51	50,5
SD	12,7	15,9	15,3	13,4	13,5	13,1	12,6	13,0

Graph I: The average results of skin hydration measurement before application (t0) and 1, 3 and 5 hours after the product application in arbitrary units.

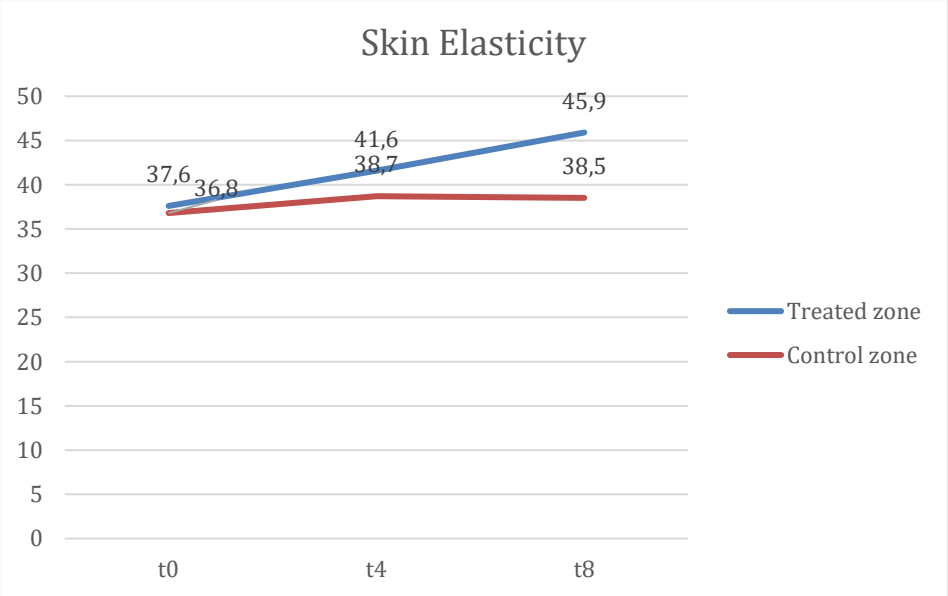


PRESENTATION OF RESULTS- ELASTICITY

Table 2: The results of skin elasticity measurement before application (t0), after 4 weeks (t4) and 8 weeks (t8) after the product application in arbitrary units. Each result is the average of 5 individual measurements

	Treated zone			Control zone		
SUBJECT CODE	t0	t4	t8	t0	t4	t8
001	27	28	30	25	29	30
002	44	46	50	45	47	47
003	29	34	38	29	27	30
004	34	38	41	37	35	36
005	46	52	60	41	51	44
006	61	67	70	60	63	61
007	49	54	62	47	48	49
008	32	34	38	31	33	35
009	38	45	50	38	38	37
010	16	18	20	15	16	16
MEAN	37,6	41,6	45,9	36,8	38,7	38,5
MIN	16	18	20	15	16	16
MAX	61	67	70	60	63	61
MEDIAN	36	41,5	45,5	37,5	36,5	36,5
SD	12,9	14,2	15,4	12,6	13,7	12,4

Graph II: The average results of skin elasticity measurement before application (t0), after 4 weeks (t4) and 8 weeks (t8) after the product application in arbitrary units.

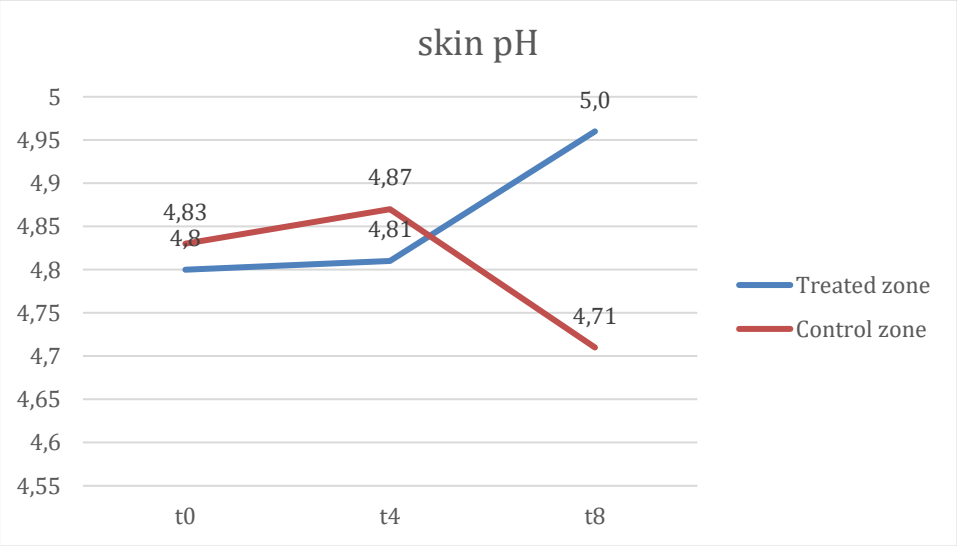


PRESENTATION OF RESULTS- pH

Table 3: The results of skin pH measurement before application (t0), after 4 weeks (t4) and 8 weeks (t8) after in arbitrary units. Each result is the average of 5 individual measurements

	Treated zone			Control zone		
SUBJECT CODE	t0	t4	t8	t0	t4	t8
001	5,0	5,0	4,8	5,0	5,0	5,0
002	5,0	5,5	5,3	5,3	5,0	5,0
003	5,0	4,9	5,1	5,0	5,2	5,2
004	4,0	4,5	4,6	4,3	4,4	3,7
005	4,0	3,9	4,0	3,9	4,1	4,0
006	4,0	4,2	4,5	3,9	4,1	4,1
007	5,0	4,8	5,1	4,9	4,9	4,6
008	6,0	5,9	6,0	6,2	5,9	5,9
009	5,0	4,9	4,9	4,9	5,2	4,6
010	5,0	4,5	5,3	4,9	4,9	5,0
MEAN	4,8	4,81	5,0	4,83	4,87	4,71
MIN	4	3,9	4	3,9	4,1	3,7
MAX	6	5,9	6	6,2	5,9	5,9
MEDIAN	5	4,85	5	4,9	4,95	4,80
SD	0,6	0,6	0,5	0,7	0,5	0,7

Graph III: The average results of skin pH measurement before application (t0), after 4 weeks (t4) and 8 weeks (t8) after the product application in arbitrary units.

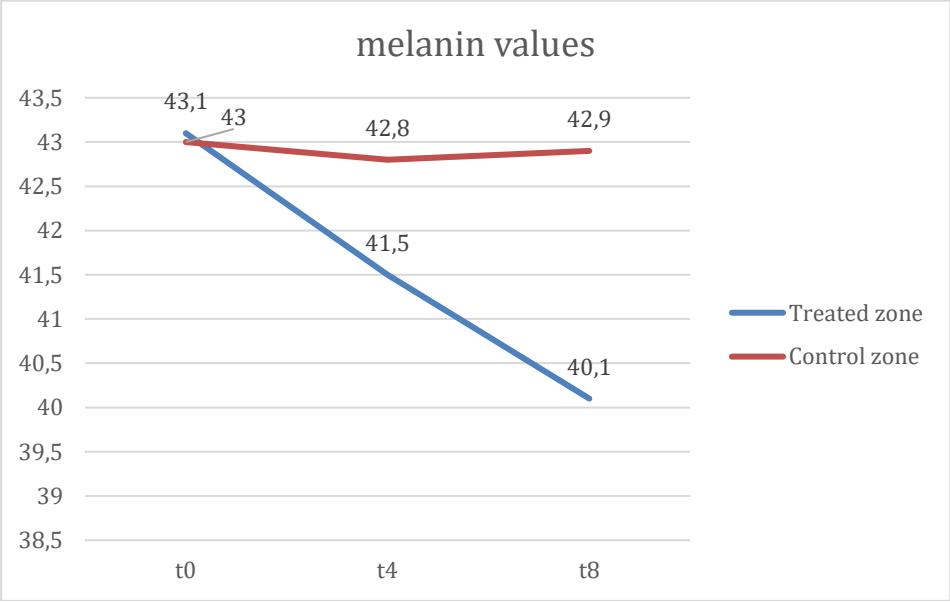


PRESENTATION OF RESULTS- MELANIN

Table 4: The results of skin melanin measurement before application (t0), after 4 weeks (t4) and 8 weeks (t8) after the product application in arbitrary units. Each result is the average of 5 individual measurements

	Treated zone			Control zone		
SUBJECT CODE	t0	t4	t8	t0	t4	t8
001	31	31	32	30	31	33
002	78	77	83	77	78	79
003	71	69	65	71	72	71
004	42	37	38	42	43	40
005	18	18	16	19	17	17
006	36	35	32	36	35	35
007	16	14	13	16	15	15
008	34	36	34	35	33	34
009	34	33	33	33	33	34
010	71	65	55	71	71	71
MEAN	43,1	41,5	40,1	43	42,8	42,9
MIN	16	14	13	16	15	15
MAX	78	77	83	77	78	79
MEDIAN	35	35,5	33,5	35,5	34	34,5
SD	22,4	21,5	21,7	22,1	22,9	22,8

Graph IV: The average results of skin melanin measurement before application (t0), after 4 weeks (t4) and 8 weeks (t8) after the product application in arbitrary units.



PRESENTATION OF RESULTS- DEPTH AND AMOUNT OF WRINKLES

Subject 1 (eye area)

Before product application	After 8 week treatment
	

Subject 2 (mouth area)

Before product application	After 8 week treatment
	

Subject 3 (forehead)

Before product application	After 8 week treatment
	

Subject 4 (eye area)

Before product application	After 8 week treatment
	

Subject 5 (eye area)

Before product application	After 8 week treatment
	

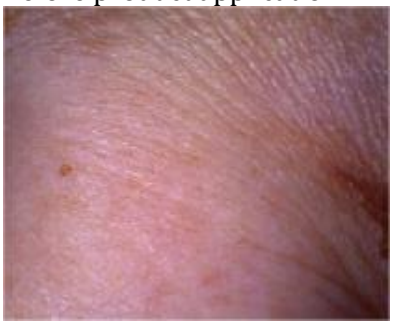
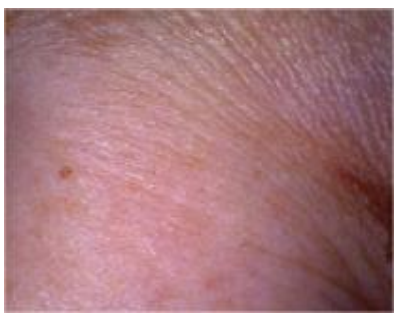
Subject 6 (forehead)

Before product application	After 8 week treatment
	

Subject 7 (eye area)

Before product application	After 8 week treatment
	

Subject 8 (eye area)

Before product application	After 8 week treatment
	

Subject 9 (mouth area)

Before product application	After 8 week treatment
	

Subject 10 (eye area)

Before product application	After 8 week treatment
	

Table 5: Assessment of product anti-wrinkle effect:

The product corrects skin elasticity	80% positive responses
The product corrects skin firmness	80% positive responses
The product improves appearance of the skin	85% positive responses
The product reduces the appearance of wrinkles	80% positive responses

6. CONCLUSION

SKIN HYDRATION EFFECT

Assumption:

The product hydrates the skin, if the parameter value increases over time.

Conclusion:

The product was found to increase skin hydration level within 1 hour, 3 hours and 5 hours after application.

SKIN ELASTICITY EVALUATION

Assumption:

The product increases elasticity of the skin, if the parameter value increases over time.

Conclusion:

The product was found to increase skin elasticity level within 8 weeks after application.

SKIN pH EVALUATION

Assumption:

The product strengthens skins barrier function if the pH values are below 5,0. This is in line with existing literature, where a relatively large number of reports (c. 50%) actually describes pH values below 5,0 as optimal; this is in contrast to the general assumption, that skin surface pH is on average between 5,0 and 6,0. Not only prior use of cosmetic products, especially soaps, have profound influence on skin surface pH, but the use of plain tap water, in Europe with a pH value generally around 8,0, will increase skin pH up to 6 h after application before returning to its 'natural' value of on average below 5,0. It is demonstrated that skin with pH values below 5,0 is in a better condition than skin with pH values above 5,0, as shown by measuring the biophysical parameters of barrier function, moisturization and scaling. An acid skin pH (4-4.5) keeps the resident bacterial flora attached to the skin, whereas an alkaline pH (8-9) promotes the dispersal from the skin.

Conclusion:

The product was found to slightly increase pH value of the skin after 8 weeks of using it once per day.

SKIN MELANIN EVALUATION

Assumption:

The product brightens the skin, if the parameter value decreases over time.

Conclusion:

The product was found to decrease melanin level within 8 weeks after application.

DEPTH AND AMOUNT OF WRINKLES

Assumption:

The product slightly reduces depth and amount of wrinkles. Both parameters are measured and determined with micro camera and in use test.

Conclusion:

The product was found to slightly reduce the depth and amount of wrinkles after 8 weeks of usage, based on the measurements and consumer evaluation of the product.

7. SUMMARY OF THE REPORT

SKIN HYDRATION EFFECT

Under the study conditions, after single application, can be concluded that the tested product hyrates the skin within 1 hour, 3 hours and 5 hours after product application.

Table 6: Values of skin hydration rate before application and 1, 3 and 5 hours after application in arbitrary units (AU) in comparison to the control zone.

TIME	Δt1	Δt3	Δt5
AVERAGE	11,6	9,8	3,6

SKIN ELASTICITY

Under the study conditions, after continuous daily application of the product, can be concluded that the tested product increases skin elasticity.

Table 7: Values of skin elasticity before application (t0), 4weeks (t4) and 8 weeks (t8) after the product application in arbitrary units in comparison to the control zone.

TIME	Δt4	Δt8
AVERAGE	2,1	6,6

pH MEASUREMENT

Under the study conditions, after eight week daily application, can be concluded that the tested product slightly increased pH value up to 5,0.

SKIN MELANIN CONTENT

Under the study conditions, after continuous daily application of the product, can be concluded that the tested product reduces skin melanin content.

Table 8: Values of skin melanin content before application (t0), 4weeks (t4) and 8 weeks (t8) after the product application in arbitrary units in comparison to the control zone.

TIME	Δt4	Δt8
AVERAGE	-1,4	-2,9

REDUCTION OF AMOUNT AND DEPTH OF WRINKLES

Under the study conditions and continuous daily application of the product, can be concluded that the tested product slightly decreased the amount and depth of wrinkles.

Product Sk.in Gloss confirmed to hydrate, increase skin elasticity, reduce melanin level and reduce the appearance of wrinkles. pH was slightly increased after 8 week usage, but the value still provides acceptable barrier function of the skin.

8. REFERENCES

- Regulation of the European Parliament and of the Council (EC) No. 1223/2009 of 30 November 2009 on cosmetic products.
- Cosmetics Europe – The Personal Care Association (previously COLIPA) Guidelines "Product Test Guidelines for the Assessment of Human Skin Compatibility 1997."
- Cosmetics Europe – The Personal Care Association (previously COLIPA) Guidelines for the Evaluation of the Efficacy of Cosmetic Products 2008.
- SOP 014 at Luamed company
- Joachim W. Fluhr. Practical Aspects of Cosmetic Testing. ISBN 978-3-642-05067-1