Received: 5 October 2020 | Revised: 12 January 2021 | Accepted: 20 January 2021
DOI: 10.1111/jocd.13965

ORIGINAL CONTRIBUTION

JCD Journal of Cosmetic Dermatology WILEY

Mechanisms of skin moisturization with hyperharmonized hydroxyl modified fullerene substance

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Abstract

Background: Hyper Harmonized Hydroxyl Modified Fullerene Substance (3HFWC$^+$) establishes hydrogen bonds with the surrounding water molecules and organizes them in clusters with the liquid crystalline state, similar to the properties of water surrounding the biomolecules.

Aims: To investigate the moisturizing properties of hyperharmonized fullerenol—3HFWC$^+$ as an emulsion O/W ingredient on the skin.

Patients/Methods: We have analyzed the reflexion of the blue light from the skin, in vivo, with different levels of moisturization and compared the influence of three groups of cosmetic products (with various active ingredients, 3HFWC$^+$ or water in same percentage in the "vehiculum") on skin moisturization by measuring paramagnetic/diamagnetic properties by Optomagnetic Imaging Spectroscopy.

Results: Regenerating Cream and Body Lotion have shown statistically significant increase of diamagnetic features predominantly in all 3 types of creams, while the Hand Cream formulations have not shown statistically significant results after 4 weeks of treatment. Anti-aging cream with 3HFWC$^+$ demonstrated statistically significant increase of paramagnetic properties of the skin (p^+/p^- from 0.90 to 0.62) and showed beneficial effects on information transfer and water content in stratum corneum.

Conclusion: Although it does not act as a classical moisturizing agent on the skin, 3HFWC$^+$ can increase moisturization as a result of hydrogen bonds established among 3HFWC$^+$ and water or biomolecules, liquid crystalline state of water in 3HFWC$^+$ resembling the properties of water surrounding the biomolecules, and finally, liquid crystalline state of water in the products.

KEYWORDS
hydrogen bonds, hydroxylated fullerene, liquid crystalline state, skin moisturization

1 | INTRODUCTION

One of the most important health-preserving and anti-aging skin care procedures involves skin moisturization.[1] Hydrated skin has a corresponding appearance, softness and complexion. Water is essential for normal metabolic activity, adequate function of biomolecules and structures of the skin, and for proper barrier function. Maintaining the skin barrier properties is of crucial importance for skin care, protection against repeated exposure to various external agents, in and out flux of various substances and rate-limiting factor for transepidermal water loss (TEWL).

In order to prevent TEWL, stratum corneum (SC) should contain 20%-35% of water.[2] There are two pivotal mechanisms that maintain equilibrium state of SC hydration in the skin[3]:

1. Water retention by the hygroscopic components of the natural moisturizing factor (NMF) in the corneocytes,[3] and
2. Controlled transcutaneous water flux. There is a water gradient in the skin, as water is provided from the inside of the body and evaporates through skin surface, this process is known as transepidermal water loss.[4] For water loss prevention, proper structure and function of skin barrier are of the outmost importance: SC lipids (ceramides, cholesterol, and free fatty acids in almost equimolar ratio) form repeated lipid bilayers positioned among the corneocytes, parallel to the skin surface.[5] Thin water layers are located in the lipid phase, around the polar head groups.[5-7] Water is able to stabilize the bilayer lipid structures, connecting the head groups with a strong network of hydrogen bonds.[8]

In human body, 40% of water is found in the form of free, bulk water, and 60% is connected to the biomolecules. Like in other epithelial tissues, water in the skin takes form of liquid crystalline, as a result of body temperature, nano level space, and strong electrical discharge in small volume. Liquid crystalline phase has different properties than free (bulk) water and enables skin softness and pliability.[6]

Recently, modified fullerene has attracted attention, due to a possibility to increase moisturization of the skin, as well as for many other positive biological functions.

Fullerene, as a third allotropic modification of carbon, beside the diamond and graphite, is a hollow, one nanometer size spheroid structure made entirely of carbon, presenting unique structure in nature with the perfect icosahedral symmetry.[9] By surface functionalization—covalent attachment of hydroxyde groups, hydroxylated fullerenes—fullerols could be produced. This modification increases its hydrosolubility and establishes connections to water molecule via hydrogen bonds. Solubility of fullerols in aqueous media depends on the number and position of hydroxyl groups.[10]

Water in the biological structures has dominant diamagnetic properties (most electrons are paired) and paramagnetic properties (due to the presence of many unpaired electrons in water clusters). There is an abundance of diamagnetic components in the skin, and only a few with paramagnetic properties (metalloproteins, melanin, free radicals, some metal ions, etc), but they are crucial for life.[11] As well as water molecules, fullerols have unique dynamic paramagnetic/diamagnetic properties. Symmetry properties of the fullerol structure determinate its vibrational and rotational energy state; total energy of C_{60} cage (translational, vibrational, rotational, and electronic) is in accordance to the icosahedral symmetry rule $\pm$ 1/2(1+√5) and $\pm$ 1/2(1-√5) (eigenvalues T_{1g},T_{2g}, T_{1u}, T_{2u}), which corresponds to Fibonacci sequences.[12] Vibrations from these structures can pass to hydrogen bonds of ubiquitous water molecules and reorganize the unnatural state of biomolecules, forcing them to oscillate and function in the natural way. When the external oscillatory magnetic field is applied, or material with paramagnetic features, like fullerols used, magnetization parallel to the magnetic field is acquired. Only the unpaired electrons can take the discrete portion of energy (quanta) and enter into a resonance.[11] Thanks to the noncovalent hydrogen bonds, water molecules pass through self-ionization process and self-organize in complex water matrix: stable, short-living clusters, water shells, and linear chains.[13] Hydrogen bonds are also important for conformation state and function of biomolecules (DNA, proteins, etc). Any change in bonding strength or its disruption can initiate change in conformation state of the molecule and in the case of proteins, for example, can provoke loss of protein functionality and disorder in the metabolic processes.[6,14,15] Thanks to this arrangement of biomolecules, dynamic properties and signal transduction are improved; skin reacts faster to the information signaling from the inside and outside and regenerates better.[16]

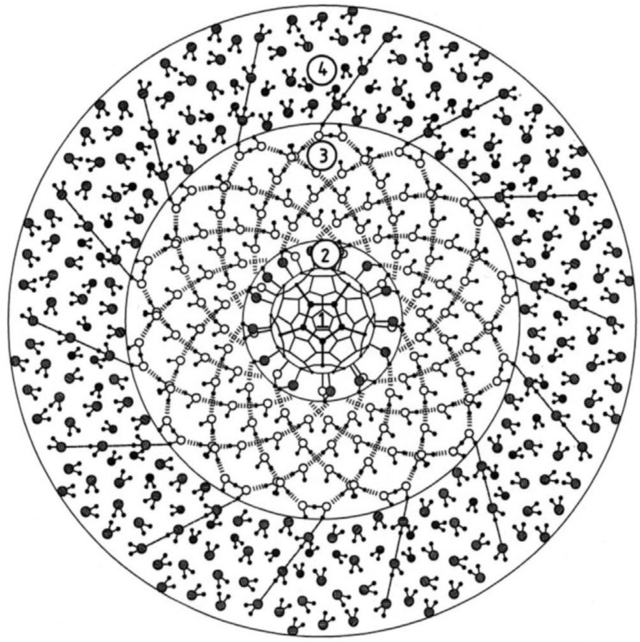


FIGURE 1 Schematic presence of the structure of 3HFWC$^+$: 1-molecule C_{60}; 2- OH groups from 12 to 48; 3- water ordered layers, from 3 to 12, by icosahedral symmetry rule, 4- ordinary water as an environment

New, patented ingredient, Hyper Harmonized Hydroxyl Modified Fullerene Substance (3HFWC$^+$) is a derivate of fullerol structure surrounded by water molecules and represents hydrosoluble structure with diameter size of 6-18 nm, without cytotoxic effects, as approved in various studies.[10,17] Harmonization provides abundance of valence electrons forming a cloud of electrons capable to create strong hydrogen bonding network. In this structure, like in many biomolecules (DNA, clathrin, collagen, microtubules etc), ratio of the length of covalent and noncovalent hydrogen bonds obeys the Fibonacci sequential rule, the same as eigenvalues T_{1g},T_{2g}, T_{1u}, T_{2u} of icosahedral symmetry ($\pm$1/2(1+√5) = $\pm$ 1.61803 = $\pm\Phi$ and $\pm$ 1/2(1-√5) = $\pm$ 0.61803 = $\pm\phi$.[12] There are three levels of hydrogen bonds, with the lowest one as the strongest one (covalent C-O-H, noted as 2 in Figure 1), and two-layered noncovalent hydrated structure (noted as 3 in Figure 1) with the formula: $\{[C_{60}(OH)_x]_y@\langle[(OH)_2]_n[(H_2O)]_m\rangle\}^{\Phi}$.

Hyperharmonized fullerol - 3HFWC^{+}[17] establishes hydrogen bonds not only with the surrounding water molecules but with

TABLE 1 Cosmetic products analyzed in this study and percentages of water/3HFWC$^+$ used in the products

Commercial products (vehiculum + WSAS) (C)	Base (vehiculum + water) (B)	Vehiculum + 3HFWC$^+$ (F)
Regenerating cream (vehiculum1 + 9% WSAS)	Vehiculum1 + 9% water	Vehiculum1 + 9% 3HFWC$^+$
Anti-aging cream (vehiculum2 + 10% WSAS)	Vehiculum2 + 10% water	Vehiculum2 + 10% 3HFWC$^+$
Body lotion (vehiculum3 + 12% WSAS)	Vehiculum3 + 12% water	Vehiculum3 + 12% 3HFWC$^+$
Hand cream (vehiculum4 + 8% WSAS)	Vehiculum4 + 8% water	Vehiculum4 + 8% 3HFWC$^+$

Abbreviation: WSAS, water soluble active ingredients.

hydrogen bonds and ions of biomolecules. Highly hydroxylated fullerene forms water chains and clusters with water molecules connected with 2-4 hydrogen bonds and bonded water.[14]

In this study, we are dealing with the properties of hyperharmonized fullerenol—3HFWC$^+$ (INCI name: water (and) hydoxylated fullerene) as an emulsion O/W ingredient hydrating the skin and its potential mechanism of skin moisturization. In order to investigate how moistrurized skin maintains equilibrium state of SC hydration in the skin, blue light has been used. Blue light is chosen because it is fifty times more sensitive to water content than green and hundred times more sensitive than red light. First part of the study dealt with the interaction of blue light with the normal skin with different moisture levels (normal, dry, moisturized and treated with the cream containing 3HFWC$^+$) using spectrophotometry, while the second part of the study dealt with the effects of different cosmetic creams, including the creams with various amounts of 3HFWC + on the skin moisturization measured by Optomagnetic Imaging Spectroscopy.

2 | MATERIALS AND METHODS

The study was conducted on 38 volunteers (female) with no dermatologic diseases and with normal to dry skin. Subject's age ranged from 31 to 69 years (mean age of the participants was 41.3). All enrolled subjects have signed written informed consent for participation in the study.

First part of the study involved 16 out of 38 volunteers with normal skin. In this part of the study, the reflexion of the blue light from the skin was measured and analyzed, in vivo, using UV-VIS-NIR spectrometer (Hamamatsu, Japan). Blue light may penetrate stratum corneum (thickness about 100 μm) and be reflected from stratum granulosum. During penetration and reflection, blue light interacts with water in stratum corneum. Although blue light has numerous side effects on the skin, in this experiment it was used only for the investigation of moisture level in the skin. Since the blue light, compared with other types of light (UV, green, red, and infrared) has the highest reflection index for water, the value of reflection of blue light was chosen to be criteria of water presence in stratum corneum.

Since the area under the spectra curve of blue light directly relates to water presence, ratio of area "*p+*," under the up curve and x-coordinate (number of pixels) and *"p-"* under down curve and x-coordinate is taken as criteria of moistening.

The 16 volunteers were divided into four groups of four participants, and we have investigated moisture level in the: normal skin (four volunteers), dry skin (normal skin of the four volunteers treated with a stream of warm air for 30 min, with 10 pauses of 1 min), moisturized skin (normal skin of the four volunteers soaked with the wet sponge for 20 min, 10 times, on every 2 minutes), and normal skin of the four volunteers treated with Anti-aging cream with 3HFWC$^+$.

In the second part of the study, we have used four commercial cosmetic products, named Regenerating Cream, Anti-aging Cream, Body Lotion, and Hand Cream (Intercosmetica, Switzerland), all of them were O/W (oil/water) type emulsions. The vehiculum of every cream was different, mostly composed of ingredients with emollient/skin conditioning/smoothing/skin protecting effects on the skin. According to the intended use of these creams, active ingredients were various herbal extracts, moisturizers, peptides, etc. We have analyzed three groups of the cosmetic products stated above: original commercial cosmetic products (Regenerating Cream, Anti-aging Cream, Body Lotion and Hand Cream) as the first group; the corresponding commercial products in which water soluble active ingredients were substituted with water (the second—placebo group of cosmetic products, named "base"); and the corresponding commercial products in which water soluble active ingredients were substituted with Hyper Harmonized Hydroxyl Modified Fullerene Substance—3HFWC$^+$ (TFT Nano Center, Serbia)(the third group). The percentages of water/3HFWC$^+$ used to substitute the water soluble active ingredients of commercial products are shown in Table 1. Different concentrations between the products were in line with the amount of water soluble ingredients in every product.

In the second part of the study, subjects were randomly assigned into one of the following three groups: 5 subjects in a placebo group, 5 subjects who applied commercial cosmetic product (with the same cream base as used with 3HFWC$^+$ substance), and 28 subjects who used cosmetic products with 3HFWC$^+$.

Cosmetic products were applied always at the same place, to the inner side of the left forearm and to the outer side of the left hand. Regenerating Cream, Anti-aging Cream, and Body Lotion were applied on the left hand positions marked 1, 2, and 3, respectively on the Figure 2, while Hand Cream was applied on the outer side of the left hand. Right hand was not treated with any product

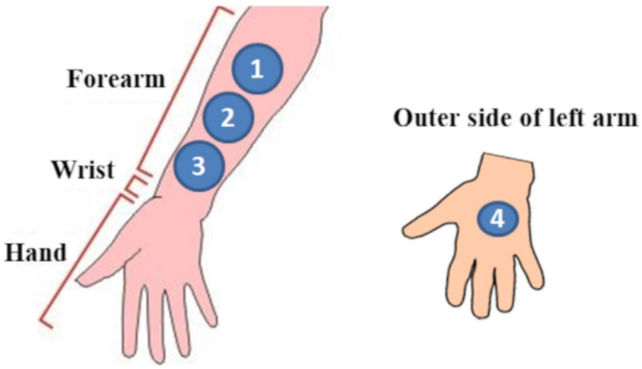


FIGURE 2 Application sites: the inner side of left forearm and the outer side of the left hand

and served as control area. Products were applied every morning, during 4 weeks.

The moisturizing properties of 3HFWC+ in the second part of the study were investigated by Optomagnetic Imaging Spectroscopy (OMIS, NanoWorld AG). Optomagnetic Imaging Spectroscopy is a method for the analysis of light-matter interaction based on spectral convolution.[18] OMIS enables collection and analysis of dermal images using a noninvasive imaging device, which includes a set of light emitting diodes serving as electromagnetic radiation source, and detector for measuring re-emitted radiation from the location on the skin.[6,13,19] This method determines a moisture level based on the ratio of incident and reflected light, by capturing an image of skin illuminated with incident white diffuse light (W), blue light (B), and angled light. By generating a normalized red and blue color channel histograms for each image and correlating the normalized color channel histograms to a wavelength scale, color channel spectral plots are obtained. Convoluting the spectral plots by subtracting the spectral plot for angled light from the spectral plot for nonangled light for each color channel, spectral signature of the skin (B-w) is generated. The spectral signature contains paramagnetic/diamagnetic properties expressed in terms of the area under the positive (p^+) and negative (p^-) peaks ratio; thus, the areas and peaks are analyzed over time in order to monitor skin state.[20] Data were statistically analyzed with *t* test in R software environment for statistical computing and graphics.

Biophysical skin properties (diamagnetic/paramagnetic) were measured by Optomagnetic Imaging Spectroscopy before any product was applied, that is, at the beginning of the study, and then repeated every 7 days in the following 4 weeks. Measurements were done under controlled conditions: temperature 22 ± 1°C; humidity 30 ± 2%, average value of environmental electric and magnetic fields: 5.82 V/m and 28.10 nT, respectively.

3 | RESULTS

Results of reflection of normal skin (NS), dry stratum corneum layer of skin (DS), stratum corneum moisturized skin (MS), and skin moisturized by Anti-aging cream with 3HFWC+ (NS + AA), for the first group of 16 volunteers, are presented in Table 2.

Difference between all states of skin is obvious from these results: the lowest area under the curve is shown in dry skin (1,509,023), and the highest in the normal skin treated with Anti-aging cream with 3HFWC+ (3,822,361): skin treated by cream gives moisture level 57% higher than the one found in normal skin. These results clearly indicate the highest level of water in the normal skin treated with the cream with 3HFWC+.

Parameter (B-w) in OMIS method used in the second part of the study represents the keratin-water interaction and surface layer state in the skin. By measuring the paramagnetic/diamagnetic properties of SC, the potential of protein (keratin) molecules from SC to transduce the signal and transfer the information can be captured. Figure 3 presents mean values before/after the treatment analyzed with blue light (B-w) and the results of statistical analysis of the obtained data.

All types of Regenerating Cream showed almost the same properties, but Regenerating Cream with 3HFWC+ and Commercial Regenerating cream (p^+/p^- from 0.62 to 0.71 and p^+/p^- from 0.64 to 0.83 – Figure 3) showed statistically significant decrease of unpaired electrons and improved diamagnetic properties.

All tested formulations of Body Lotion have shown the statistically significant increase of diamagnetic features (p^+/p^-): from 0.27 to 0.74 (B); from 0.40 to 0.79 (C); from 0.57 to 0.73 (F) (Figure 3).

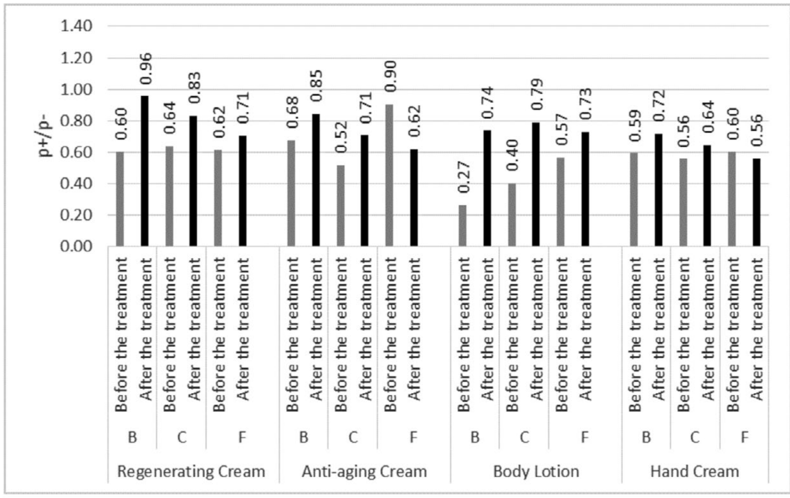
None of the formulations of Hand Cream has shown statistically significant results: $P = .055$ (B); $P = .382$ (C); $P = .165$ (F).

TABLE 2 Reflection of blue LED with peak at 431.4 nm: normal skin, dry skin, stratum corneum moisturizing skin, and skin moisturized by Anti-aging cream with 3HFWC+ for the first group of 16 volunteers

Blue light (431.4 nm)					
	LED	Normal skin (NS)	Dry skin (DS)	Moisture skin (MS)	NS + Anti-aging cream
Peak intensity (a.u.)	61,338.3	57,781.4	55,795.6	59,727.3	62,231.6
Wavelength of peak (nm)	431.4	463	463	463	463
Area (pixels)	2,119,697	2,178,762	1,509,023	3,574,729	3,822,361

Abbreviation: a.u., arbitrary units.

FIGURE 3 Comparison of p+/p- values before (gray) and after (black) the treatment of 3 groups of cosmetic products (B - vehiculum + water; C-commercial products; F-vehiculum + 3HFWC+) and the results of statistical analysis of the obtained data



Regenerating Cream			Anti-aging Cream			Body Lotion			Hand Cream		
t-test p value											
B	C	F	B	C	F	B	C	F	B	C	F
0.063	0.007	0.001	0.077	0.079	0.0001	0.003	0.004	3.91E-05	0.055	0.382	0.165
*			*	*					*	*	*

B - vehiculum + water; C-commercial products; F-vehiculum + 3HFWC+
**p> 0.05, results are not statistically significant*

Anti-aging cream with 3HFWC+ showed statistically significant results: p^+/p^- from 0.90 to 0.62. Other two tested creams in this group have not showed statistically significant results: $P = .077$ (B); $P = .079$ (C).

4 | DISCUSSION

Decrease of p^+/p^- ratio value in the (B-w) spectra after the treatment indicates higher number of unpaired electrons and an increase in paramagnetic properties.[6,21] Thanks to the abundance of unpaired electrons, 3HFWC+ establishes hydrogen bonds which enable energy transfer and signal transduction, it organizes water molecules in clusters and shells and improves hydration of the SC. In the case of higher level of p^+/p^- after the treatment, diamagnetic properties are increased, tissue becomes more stable with higher density, lipid layers are highly ordered and more compact, keratin fibers are less swollen, and TEWL is decreased. The 3HFWC+ makes optimal length and structure of lipid segments in SC, water passing time is longer, and skin barrier function is better.

4.1 | Regenerating cream

Regenerating Cream with 3HFWC+ and Commercial Regenerating cream (p^+/p^- from 0.62 to 0.71 and p^+/p^- from 0.64 to 0.83 – Figure 3) showed statistically significant decrease of unpaired electrons and improved diamagnetic properties (Figure 3). These changes enable tissue stability and proper lipid layers ordering and density. These formulations could improve skin hydrating properties by preventing water loss to the atmosphere.

4.2 | Body lotion

All tested formulations of Body Lotion have shown statistically significant increase in diamagnetic features (p^+/p^- from 0.27 to 0.74) (Figure 3). The number of unpaired electrons is decreased, and tissue dynamics is diminished. SC becomes a compact structure with stronger barrier properties. These products prevent water loss by forming semipermeable structures on the skin surface and at the same time, protect skin from harmful external influences.

However, both of these products can influence skin moisturization even if there is a decrease of paramagnetic properties. Since C_{60} cage behaves as a nano-generator of electrical and magnetic fields, formulations with 3HFWC+ can improve hydration of SC, thanks to the hydrogen bonds and liquid crystalline state of water layers. Ueoka and Moraes showed similar results[22] and concluded that formulations that form liquid crystalline are usually more chemicophysically stable and better promote skin hydration by forming films on the stratum corneum. Other studies have also demonstrated that liquid crystalline phase creating can improve the formulation by increasing water retention in the SC, which increases hydration and permeability.[22] In the case of tested formulations, they improved hydration and protect skin by forming a liquid crystalline layer on the surface of the SC.

4.3 | Hand cream

From the Figure 3, it can be seen that none of the formulation of Hand Cream has shown statistically significant results after 4 weeks of treatment and influenced the moisture level in the skin. We assume the interaction of the vehicle ingredient(s) and the $3HFWC^+$ in Hand cream F has the major influence on the amount and behavior of unpaired electrons. This subject will be our next field of investigation, in order to select the proper ingredients able to significantly support the moisturizing capacity of the $3HFWC^+$ ingredient.

4.4 | Anti-aging cream

Anti-aging cream with $3HFWC^+$ showed statistically significant changes in SC properties (Figure 3) and enhancement in number of unpaired electrons and paramagnetic properties, p^+/p^- from 0.90 to 0.62 (Figure 3).

Thanks to increased paramagnetic properties, $3HFWC^+$ creates the network of hydrogen bonds and water molecules in water layers with water of keratin molecules and lipid layers in SC, transfers information, keeps the liquid crystalline formations, and acts as a moisturizer.

Moisturizers found in cosmetic products are able to increase the water content of the skin acting as a humectants (glycerin), emollients (like natural oils), or as an occlusive agents (petrolatum).[2,3] The humectants attract water from the viable layers of the skin, and emollients fill in the tiny spaces between corneocytes in the superficial layer of SC, making semipermeable barrier which controls water movement in and out of the skin. The proper selection of the ingredients with the emollient effect, together with the corresponding microstructure of the product could significantly increase the moisture of the skin.[23] However, $3HFWC^+$ does not use any of these classical moisturizing mechanisms by which cosmetic agent act on the skin. We can postulate that mode of action is specific only for the $3HFWC^+$, and based on the following:

1. Hydrogen bonds established among $3HFWC^+$ and water molecules, that keep water clusters and water shells stable. Through signal transduction and energy transfer, these hydrogen bonds and water molecules organization can rearrange unnatural state of biomolecules and/or protect the proper conformational state and function of biomolecules. $3HFWC^+$ acts biophysically, by adopting the different lengths between O and H atoms in hydrogen bonds and water molecules, pushing the parts of biomolecule to change their conformational state. Among other things, it could enable molecules to open their receptor places and make them functional again,
2. Hydrogen bonds established among $3HFWC^+$ and biomolecules, that again, through signal transduction and energy transfer, improve their conformational state, hydration level, and finally, proper functions,
3. Liquid crystalline state of water in $3HFWC^+$ characterized with similar properties to water predominant in the body, surrounding the biomolecules, that enables its recognition and utilization, and
4. Liquid crystalline state of water in the products, which forms film and promotes hydration and water retention on and in the SC.

In $3HFWC^+$—hyperharmonized fullerenol C_{60} (Figure 1), numerous hydrogen bonds are attached to the surface of C_{60}, keeping the layer of water molecules, and creating stable structures—water shells. As classical/quantum moiety, hydrogen bond will establish the electrical attractions between different charges in neighboring molecules (classical) and share electrons between the hydrogen bond and stronger, covalent bond in water molecules (wave function—quantum properties). In these shells, water molecules take form of liquid crystalline, as well as the water which surrounds the biomolecules. According to a coding approach, optimal organization of water molecules is polyhedral structure of water clusters with hydrogen bonding chain network and they both are compatible with coding system of genetic code (DNA and proteins).

Thanks to the abundance of water molecules and their organization in clusters and shells, $3HFWC^+$ shows an influence on the conformational state of protein molecules, like keratin, in SC. $3HFWC^+$ produces oscillating magnetic field which causes dipole-molecules, like water or proteins to arrange in line with it, spreading this process through "domino effect," according to the icosahedral symmetry law. Under the influence of $3HFWC^+$ vibrations and signal transduction, biomolecules in unnatural state will change the conformation state and allow the water molecules as well as biomolecules (enzymes, NMF ingredients, etc) to connect and induce their proper function. This arrangement will restore the moisturizing level of biomolecules.

$3HFWC^+$ can influence the barrier function of the skin as well: water layers located around the polar head groups in lipid layers are enlarged, making more stable bilayer lipid structures[8] and changing the permeability of various agents into the skin or out of the body.[24]

It was established that fullerene, although with a diameter range of 1 nm only, cannot pass lipid layers in SC, especially the water layers with polar head groups of lipids inside the lipid membrane.[25] Different behavior of fullerene C_{60} was observed, depending on the concentration, and the permeability of fullerene molecules depends on their free energy of permeation and diffusivity.[25] Investigations examining the translocation of hydroxylated fullerene in plasma membrane showed that highly hydroxylated fullerene was usually attached to the surface of the membrane by hydrogen bonds. It was able to penetrate deeper into plasma membrane too and increase membrane fluidity. Highly hydroxylated fullerene was responsible for ion transport, permeability, membrane enzymes activity, etc.[15] It was assumed that fullerol present in polar region of phospholipids provokes lipids separation in lipid bilayers and even disrupts the order of acyl chains causing increased membrane fluidity, depending on the fullerol concentration.[15] Based on these conclusions, we could say that $3HFWC^+$ probably penetrates into SC lipid bilayers, increases water content in polar head region between the bilayers, and intrudes their ordered structures. It is important to emphasize that ceramide-molecules in lipid bilayers are able to form inter- and intramolecular hydrogen bonds, while cholesterol molecules can form only intermolecular hydrogen bonds. As $3HFWC^+$ is capable to establish hydrogen bonds, it is possible that it makes connections through hydrogen bond network with the surrounding ceramide and cholesterol molecules in lipid bilayers and creates a "relaxed" structure and more random lipid order. These effects could change the permeability of SC and influence the efficacy of skin barrier, as well as improve aesthetic appearance of the skin. This will be an object of our further investigations.

In the case of Anti-aging cream with $3HFWC^+$, apart from the surface effects of cream consisted of liquid crystalline-forming ingredient, it is evident that it can improve moisturization of the SC and transfer of the signals, as well as enable SC to resist to various external and internal influences. Improved hydration of SC will reduce discomfort of dry skin and give better tonus and consecutively better aesthetic appeal of the skin, softness and elasticity. Beside the aesthetic improvement of the skin, this effect of $3HFWC^+$ on SC hydration could be used to increase efficacy of topical and transdermal delivery drugs.

Among investigated formulations, the vehiculum of Anti-aging cream with 3HFWC + has the best influence on skin hydration, which is in agreement with the results of the experiment in the first part of the study.

5 | CONCLUSION

Hyper Harmonized Hydroxyl Modified Fullerene Substance ($3HFWC^+$) in corresponding vehiculum is promising new ingredient in cosmetic products, with moisturizing and other resulting positive effects, being efficient on the level of hydrogen bonds and conformational state of the biomolecules. Investigation with the blue light (diffuse and polarized) showed that hydration of skin (*stratum corneum*) increases for 57%, when skin is treated with Anti-aging cream with $3HFWC^+$ substance, compared with normal skin. According to the cosmetic study on 38 volunteers, products with the $3HFWC^+$ (Regenerating Cream, Anti-aging Cream, and Body Lotion) showed significant increase, more than 64% in average, of skin moisturization.

ACKNOWLEDGMENTS

We are grateful to Intercosmetica (Switzerland) who provided creams with $3HFWC^+$ substance.

CONFLICT OF INTEREST

On behalf of all authors, the corresponding author states that there is no conflict of interest.

ETHICAL APPROVAL

The study follows the tenets of the Declaration of Helsinki.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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How to cite this article: Miljkovic S, Jeftic B, Stankovic I, Stojiljkovic N, Koruga D. Mechanisms of skin moisturization with hyperharmonized hydroxyl modified fullerene substance. *J Cosmet Dermatol*. 2021;00:1–8. https://doi.org/10.1111/jocd.13965