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The Meaning of Nano Dimension Involving Cosmetics: From the Lab to an Industrial Green Process

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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Review Article

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ABSTRACT

Cosmetology has made substantial progress in the last thirty years formulating advanced cosmetics: from cosmeceuticals, nutricosmetics and neurocosmetics to specialized medical devices able to generate a *mind-body* wellness.
A comprehensive study for lab-designed innovative and green cosmetic product ready to be industrially processed will be reported and described.
The adopted formulation is based on the use of block co-polymeric nanoparticles made by the use of Chitin Nanofibrils (CN) and hyaluronic acid as raw materials. At this purpose, physiochemical biodegradability and safeness data on CN will be reported together with the preparation, characterization, and control of the final nanoemulsions used as base for the final formulation.
Some data of safety, tolerability and effectiveness for the lab-designed formulation will be also reported.
CN, used as active ingredient binding natural polymers to form block polymeric nanoparticles has been also reported together with the methodologies to produce advanced medications by electrospinning, and innovative food packaging by casting.

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The obtained data showed the possibility we could have by using nanotechnologies, especially when the selected raw material are classified as natural and obtained from waste materials, as CN is. Finally it has been showed the possibility to use the same raw material for producing cosmetics, advanced medications, and food packaging thus opening new and unsuspected *roads* to increase the industrial turnover of any cosmetic company.
In conclusion, to succeed into the market, it is necessary to carry on a formulation that, well studied and designed at lab level, produced at the right cost, controlled *in vitro* and *in vivo* to demonstrate its safety and effectiveness, should be distributed by an efficient marketing plan.

Keywords: Chitin nanofibrils; hyaluronic acid; green cosmetology; skin aging; nanoemulsion.

1. INTRODUCTION

Cosmetology has made a substantial progress in the last 30 years designing and formulating innovative cosmetics by different concepts and ideas, starting from cosmeceuticals, nutricosmetics, and neurocosmetics with a *mind-body* activity, for arriving to specialized medical devices, designed to bettering the human way of living [1-3]. More technologies and materials are ready to help the consumer to ameliorate her/his wellbeing, but many issues need to be addressed to achieve a successful outcome. Therefore, science-as-a-process rather than science-as-a-result should be necessary to formulate effective cosmetic products [4-6]. Thus the necessity to deepen the knowledge of skin's physiology and biology, as well as of physicochemical and structural activity of cosmetic active ingredients and carriers used, because of their interaction with skin layers and cells. Thus, *Nanobiotechnology,* as convergence of *Nanotechnology* and *Biotechnology* could form the basic building blocks for innovative cosmetic products, founded on the use of biomaterials instead of fossil-based ingredients [7]. This branch of science is a multidisciplinary field involving not only cosmetic chemistry, but also material engineering, mechanical and biomedical engineering, molecular cell biology, histology, clinical medicine, and naturally regulatory affairs, business administration, and commercialization transition. Consequently, study and design of innovative cosmetics, require an interactive approach with many specialists, complementary and in coordination with different disciplines [8]. The rapid prototyping of biomaterials and technology solutions into innovative cosmetic products represents, in fact, an essential business requirement for success in the marketplace, especially in this period of financial crisis. Naturally, a well designed biomaterial could have the ability to orchestrate desirable biological effects and to degrade without leaving undesirable effects [9]. At this purpose, biomolecules and nanobiotechnologies just used or in developing at level of laboratories and even in the patent portfolio of many universities and companies, could be ready to meet and marry the right complementary critical partners for having success. In any way, it exists the necessity to develop innovative nanoemulsions or biomolecules in terms of mechanism of action, biological capability and efficacy at level of human skin [10], as well as it is also indispensable to look at the real motivations necessary for changing ingredients from the existing formulations to the new ones required. In addition, there is room for changing technology by the use of *smart* biomaterial options, instead of looking for a single-technology ingredient solution, having an open mind on the new *regulatory problem* regarding, for example, the recent *no animal testing* for the ingredients of cosmetic use [11].

But what smart material means? It is defined as a material in which a key property could be altered in a controlled manner in response to the introduction of a predetermined external stimulus [12]. Therefore, stimuli-responsive material/carriers, such as Chitin Nanofibril-Hyaluronan (CN-HA) block polymeric nanoparticles, might be utilized, as proposed by this paper because of their capacity to undergo such changes as shape, mechanical rigidity/flexibility, opacity, porosity, and entrapping facility for the designed ingredients, to bettering their effectiveness and safeness [13,14]. Moreover, the carrier based on CN-HA may be not only in the form of specialized nanoemulsions and gels, but also of fibers to make advanced medications and food packaging films.

Last but not least, it seems important to use green ingredients and energy derived from renewable biological resources [15-17]. These *ingredients* that are, recyclable, renewable, and

triggered biodegradable, as CN-HA is, may reduce the dependency on the fossil fuel to a great extent, contemporary reducing the greenhouse gas emissions. Currently, attempts are being made to promote agricultural biomass and fishery's waste for producing biomaterials to be used in different fields, such as pharmaceutical, cosmetic and food [18-21]. This is why sustainable systems of production and consumption need transformative changes responding to societal challenges such as, climate modification, natural resource scarcity, and environmental pollution [22]. Thus, an economy based on plant and animal by-products, represents a significant shift in socio-economic, agricultural, industrial, energy and technical systems [23-25].

Potential benefits from the transition to a bio-based economy include a reduction of greenhouse gas emissions with a decrease in dependence on fossil resources, wiser management of natural resources, and improved goods security [25,26]. This is the reason to use CN as basic and natural raw material at nano-dimension.

2. AIMS

The aims of this paper is to try to give a rationale for lab-designed innovative and *green* cosmetic products, ready to be industrially processed by sustainable technologies. This topic is reported, described and discussed through 6 different steps.

2. STUDY DESIGN

2.1 Step 1: Preparation and Control of Chitin Nanofibrils and its Complexes, as Green Raw Material

Chitin Nanofibrils (CNs) have been used by our group as new raw material for innovative cosmetic products, advanced medication and food packaging [27-32]. This natural, non toxic polymer [33], produced from crustaceans' waste and made of 18-25 molecules of glucosamine and acetyl-glucosamine, has the same backbone of hyaluronic acid (HA) (Fig.1). The productive process of CN, made at acid pH, is characterized by a safe and ecological methodology that brings the formation of a stable water suspension containing about 300 billions nano-crystals per millilitre. During the process a secondary solution rich in glucosamine, acetyl glucosamine and oligomers are produced useful to give shine and manageability to hair. All the productive process is without final residues with a low consume of water and energy.

CNs, industrially produced by a *green patented* process [34], represent the purest crystal form of chitin, that in their dry form appear at SEM as needles of a medium dimension of 240x7x5 (Fig. 2) and, in water solution at pH between 2 and 4, are prevalently covered by positive charges. As shown on (Fig. 3) the pure quality of the obtained CN crystal are evidenced from the superior quality of the infrared spectrum (Elmer Spectrometer GX FT-IR with MCT-SL Detector), compared to other published commercial chitin, in particular at level of 1375, 1155, 953 and 896 bands. These data are confirmed by the high intensity of the peak of X-ray diffraction spectrum (Bruker AXS Detector Diffraction System), corresponding to d= 9.624 and by the presence of detailed minor peaks at other/d-values, all indicative of high regularity along the fiber axis (Fig. 4) [35].

2.2 Step 2: Preparation and Control of Block Copolymeric CN-HA Nanoparticles

It is interesting to underline that ~ 15.000 amino groups/ positively charged, covering the surface of each CN crystal, have given the possibility to produce the block copolymeric CN-HA nanolamellae and nanoparticles (Fig. 5) by the use of gelation method and the negatively charged hyaluronan [14,32,36].

The process consists of mixing the chitin nanofibril suspension at 2% (h/w) to a 2% suspension of hyaluronan at ph 7, by a high speed stirrer. The obtained lamellae homogenized, filtered and washed by a distilled water are spray dried to obtain nanoparticles as reported on (Fig. 6).

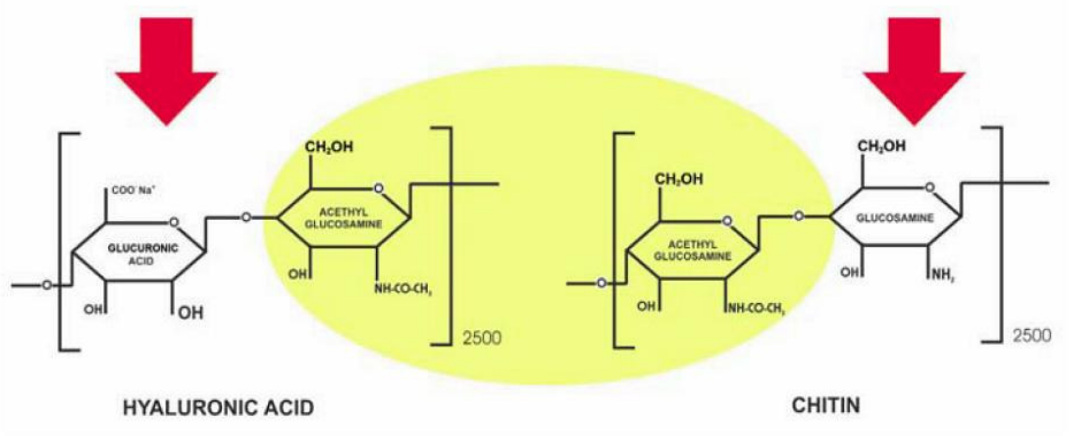

Fig. 1. Chitin has the same backbone of hyaluronic acid

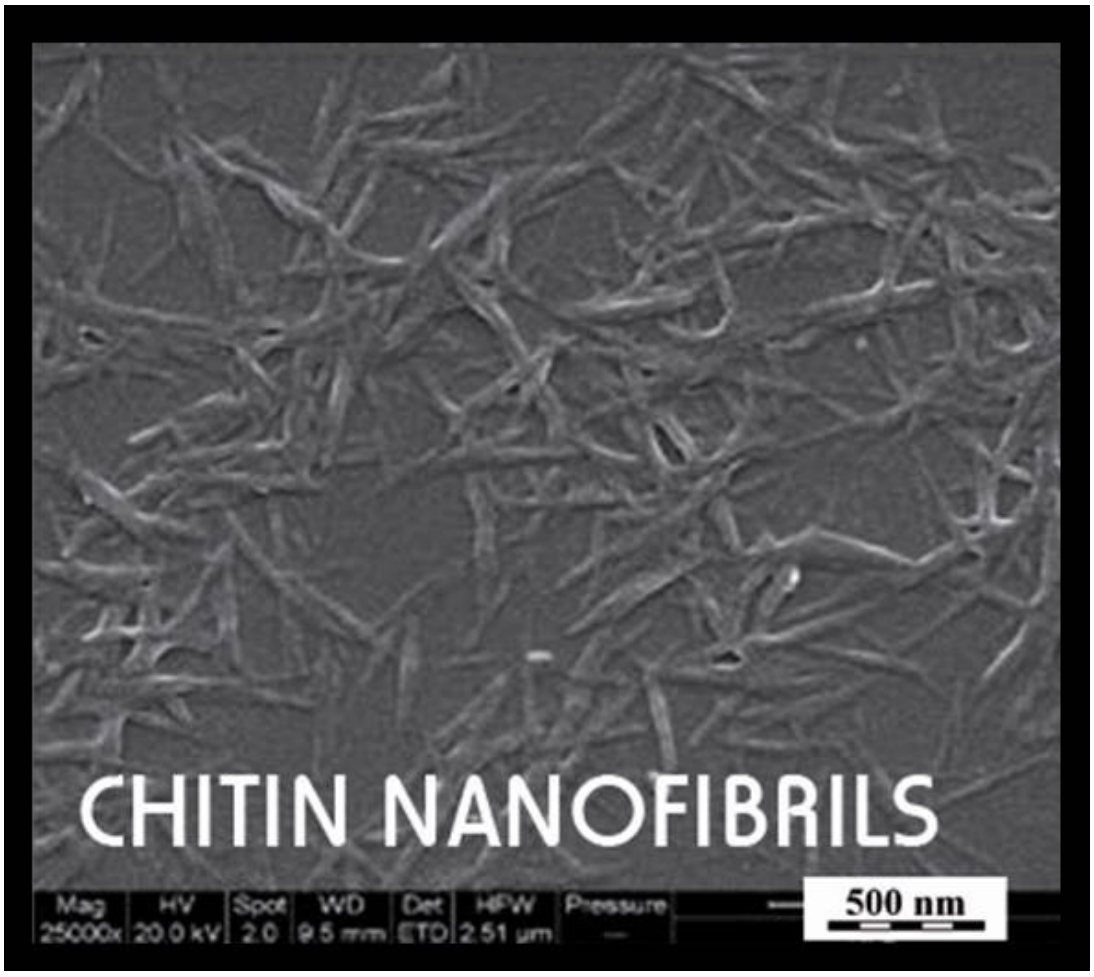

Fig. 2. Chitin nanofibrils at SEM

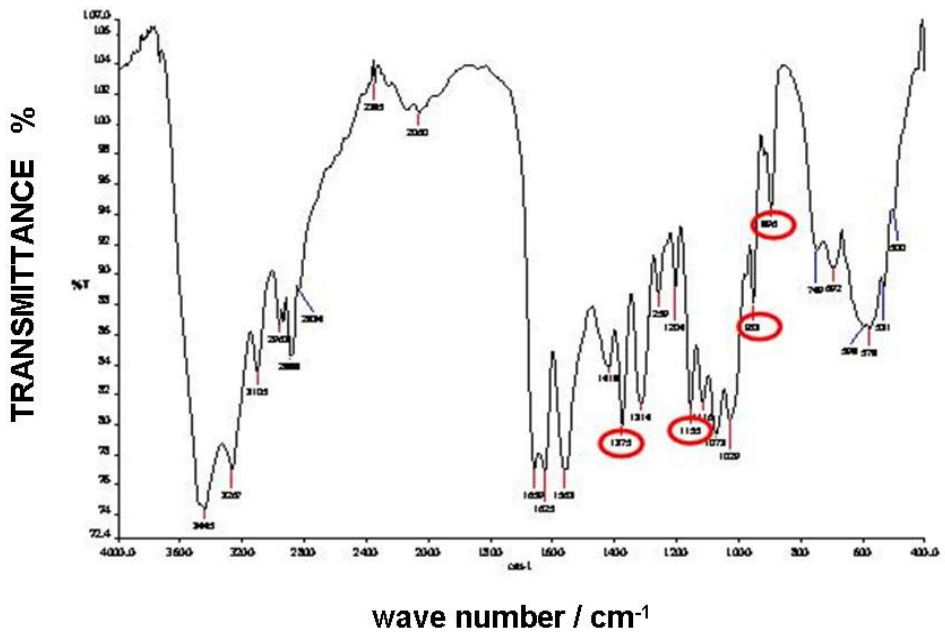

Fig. 3. Chitin nanofibril crystal form, compared with the commercial chitin, has shown a superior quality as evident by the obtained Infrared bands 1375,1155 (FT-IR spectrum)

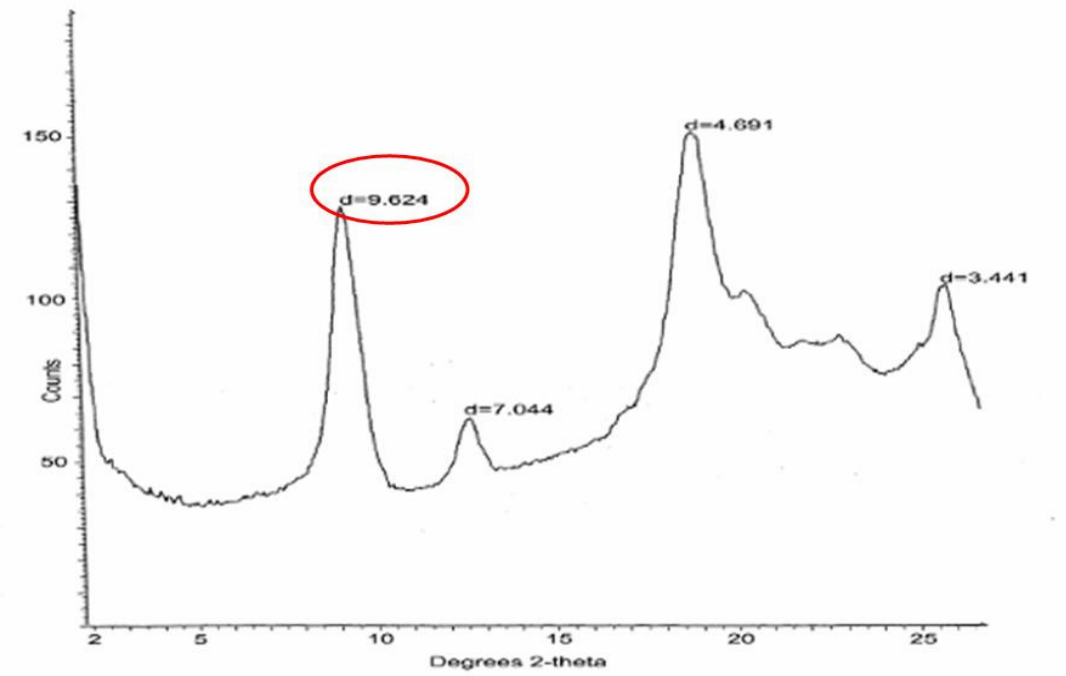

Fig. 4. Chitin nanofibril crystal form, compared with the commercial chitin, has shown a superior quality as evident by the high intensity of the 9.624 X-ray diffraction spectrum

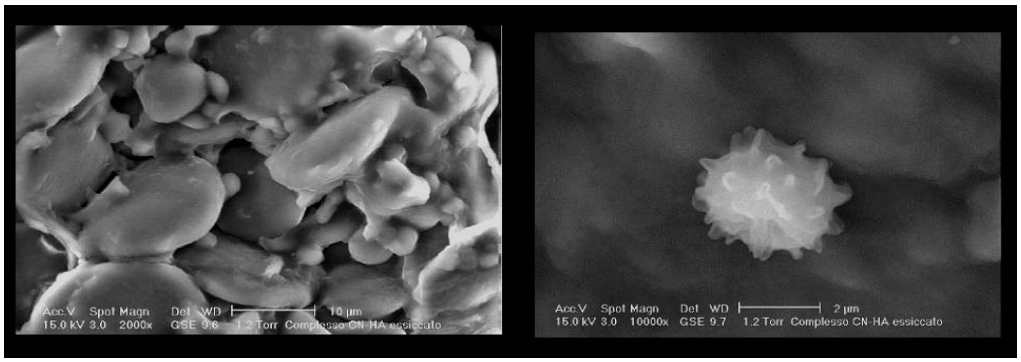

Fig. 5. Nanolamellae and nanoparticles of CN-HA at SEM

These bioactive, safe and biodegradable nanoparticles with a medium size between 250 and 400nm as appear at SEM (SEM/EDY, Phylips XL30) depending upon the nature and molecular weight of the ingredients selected and the method of preparation, may entrap different active ingredients [36-38].

It is interesting to underlining that these nanoparticles have the possibility to easily and regularly release the encapsulated ingredients at different times, as shown with lutein (Fig. 7) [37-40]. It has been also evidenced how the entrapment efficacy, the loading content and the relative release of the active may depend on the crystallinity, size and electrical charge covering the CN surface (Table. 1) [37-39]. The positive charges, in fact, seem to have the interesting ability to disturb the tight lamellar layers of the Stratum Corneum, enabling a better diffusion of the entrapped active compounds through the skin, as shown on (Fig. 8) by the stripping method [37-42].

2.3 Step 3: Biodegradability

About the environmental problem of biodegradability, as previously reported and shown from Eide KB et al. [43], Humans contain many families of the enzyme *chitotriosidase* (chitinases) capable to hydrolize both chitin and its derivatives until carbon dioxide and water [37-42]. These data were confirmed by measuring the weight variation of CN commercial chitin and *Chitosan* complexed with HA, after the enzymatic hydrolysis obtained by the activity of different enzymes, as (Table 2) [35,42].

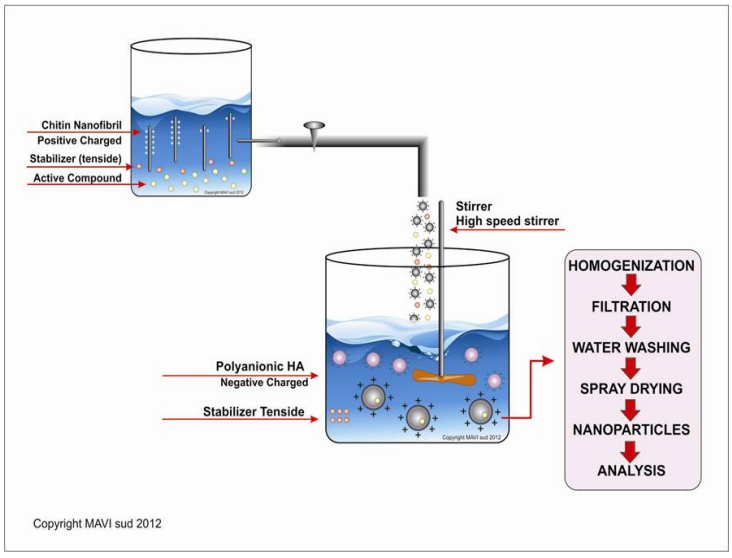

Fig. 6. Production of the CN block-polymer nanoparticles by the gelation method

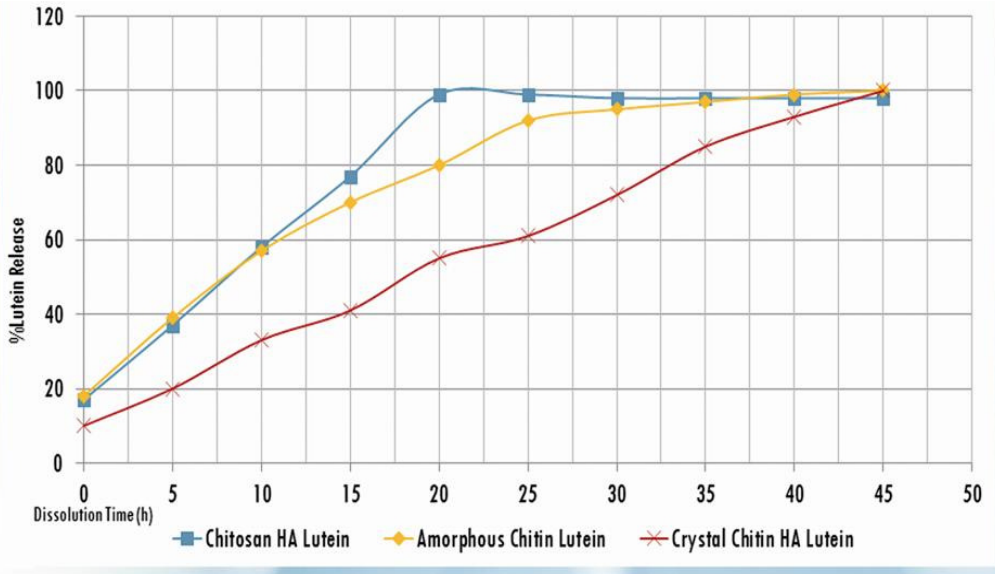

Fig. 7. Release profile for lutein from chitin/chitosan nanoparticles

Table 1. Nanoparticles yield, lutein loading content and entrapment efficiency of different kind of chitin and chitosan complexed with hyaluronic acid

Polymer	Ng nanoparticle yield (%)	Ng lutein loading content (%)	Entrapment efficacy (%)	Particle mean size (nm)
Chitosan-HA-Lutein	33±9	10±3	32±5	458±14
Amorphous Chitin-HA-Lutein	31±10	18±3	40±5	355±13
Crystal-Chitin-HA(CN)Lutein	42±9	35±3	66±6	185±13

Note: alla measurements were performed in triplicate. Abbreviations: CN= Chitin Nanofibrils; HA = Hyaluronic Acid

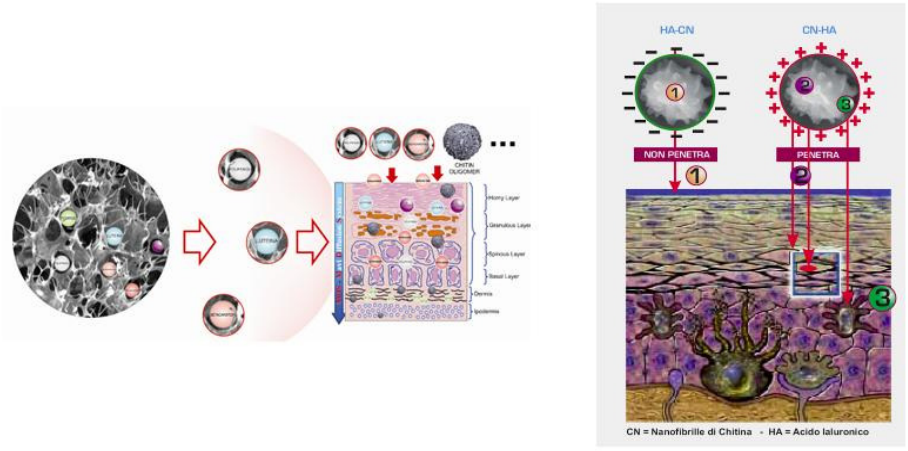

Fig. 8. CN-HA has the capacity to disturb the stratum corneum lamellae organization, increasing the skin penetrability of the active ingredients entrapped

Table 2. Weight loss of different synthetized nanoparticles upon treatment with enzymes for 48 days at 25C°

Enzyme	Crystal chitin (CN) HA lutein nanoparticles (CN)		Amorphous chitin-HA-lutein nanoparticles		Amorphous-chitosan HA lutein nanoparticles	
	Weight, g		Weight, g		Weight, g	
	Initial	Final	Initial	Final	Initial	Final
Cellulase	0.10054±0.00011	0.10053±0.00007	0.10070±0.00009	0.00033±0.00009	0.10040±0.00006	0.10020±0.00004
Pectinase	0.10034±0.00009	0.00030±0.00006	0.10040±0.00007	0.00020±0.00004	0.10045±0.00004	0.10020±0.00002
Amylase	0.10018±0.00007	0.00010±0.00009	0.10050±0.00005	0.00010±0.00003	0.10058±0.00005	0.10018±0.00003
Lysozime	0.10025±0.00009	0.00005±0.00001	0.10050±0.00009	0.00015±0.00001	0.10055±0.00005	0.10015±0.00003
Collagenase	0.10046±0.00010	0.00030±0.00005	0.10060±0.00008	0.00024±0.00007	0.10060±0.00007	0.10030±0.00005

All p values are highly significant (p … 0.005) vs initial and weight for all the enzymes tested; Note: all measurements were performed in triplicate.; Abbreviations: CN=Chitin Nanofibrils; HA = Hyaluronic Acid

2.4 Step 4: Preparation, Characterization and Control of the Nano-emulsions

Nanoemulsions (NEs) are fine O/W (oil in water) or W/O (water in oil) dispersions, having small droplets covering the size range of 20-500 nm [44-46]. Because of their small droplet size they offer increased physical and kinetic stability. The Brownian motion prevents, in fact, sedimentation or creaming of the emulsion. However, also if NEs require stabilization with emulsifying, and homogenization, their long-term physical stability makes them unique [47-49].

In any way, to make stable emulsion reproducibility, a large number of factors must be controlled such as, an accurate selection of the ingredients and surfactants used, their order of addition, the right shear stirring, the energy input, and the solubility of the oil phase in water phase and vice versa. For delivering the active ingredients entrapped into CN-HA nanoparticles (as Lutein) and inserted into NEs, on one hand it has been minimized the total surfactants used to reduce the possible side effects on the skin of the final product tested; on the other hand it has been maximized the concentration of the lipid-soluble ingredients (internal phase) in the final system, for increasing their effectiveness and obtaining a more easy penetrability through the skin layers [49,50].

The reason to use NEs for application in innovative healthy cosmetics has the following advantages:

a) They can be made using lower surfactant concentration;
b) The small size of the droplets for cutaneous use allows to deposit them uniformly on skin;
c) They are suitable for efficient delivery of active ingredients because of their high surface area and low interfacial tension ;
d) They can easily penetrate through the *rough* skin surface, enhancing penetration of *actives,* thanks to their small size;
e) The fluidity nature of the system, as well as the absence of any thickener agent may give them a more pleasant and aesthetic character and skin feel. For all these reasons we designed to use the NEs.

2.5 Preparation

The designed nanoemulsions were prepared using the spontaneous emulsification mechanism by the use of low-energy emulsification technique, accurately selecting in advance the natural-based active ingredients, the oil viscosity and the surfactants of the system [51,52]. Practically it was used the classic phase-inversion temperature method of Shinoda and Saito modified for our necessities, according also to the method of Bouchemal et al. [47,52-55]. At low temperature the surfactant monolayer causes oil swollen droplets (O/W emulsion),while at high-temperature it forms water-swollen droplets (W/O emulsion). This can be achieved by changing the temperature of the system, forcing a transition form from a O/W emulsion at low temperatures to a W/O emulsion at higher temperatures (transitional phase inversion). During the emulsion cooling period, finely dispersed droplets are formed. Moreover to further stabilize the emulsion and decrease the size of nanoparticles, contemporary increasing the droplet size distribution and the emulsion stability, a nozzle-based homogenizer was used. This operation was done at low pressure and energy consumption, before and after the addition of CN-HA nanoparticles entrapped by the different active ingredients designed to use.

2.6 Characterization and Control

The droplet size distribution of the final emulsion was measured by a diffusion method using the light-scattering particle size analyzer (LS250 Beckman), while CN-HA mean size, width distribution and zeta potential were determined by a Zetasizer (Nano ZS model Zen 3600, Malvern Instruments, Worchestershire, UK), on samples dried by the Buchi Mini B-190 spray drier (Flawil, Switzerland) and re-suspended in distilled water. The Scanning Electron Microscope (SEM/EDY, Philips XL30) was used to physically control their relative size [13,36-41].

The obtained results are reported on (Figs. 5 and 9).

2.7 Step 5: Safety, Tolerability and Effectiveness

Chitin, *Chitosan*, oligosaccharides, from fishery's waste as well as cellulose, lignans, xantan gum, and other plant derivatives from vegetal biomass are extensively used as drug and cosmetic ingredients because of their high biocompatibility and non toxicity [56-64]. However, in order to control the possible interaction among the vehicle (nanoemulsion), the different CN

nanoparticles used, and the skin, it is necessary to verify *In vitro* and *In vivo* some specific parameters that appear on wrinkled and/or photoaged skin. These controls are necessary to verify both safeness and effectiveness of the cosmetic treatment necessary to maintain the skin in good conditions or remove/repair some skin damages. This is the principal goal of cosmetic products. The solution is to develop topical systems that, delivering the active ingredients to the different cell layers of the viable epidermis, could penetrate the horny layer barrier and give benefits without disrupting their structures or having pharmacological activities. Among the galaxy of the *in vitro* methods to control efficacy and tolerability of an antiaging product, the following were selected for our studies and recovered on keratinocyte and fibroblast cultures. Thus, by the use of CN-HA nano particles, entrapping different active ingredients it was possible to show: (a) the control of the pro-inflammatory cytokines by the IL-8 release (Fig. 10), (b) the metalloproteinase release (Fig. 11), (c) the inhibitory activity of collagenase (Fig. 12), as well as (d) the antioxidant activity by the TRAP method (Fig. 13), (e) the synthesis of collagen IV (Fig. 14), (f) the increase of the chaperon HSP47 (Fig. 15), as reported and described in reference 65.

The data reported in (Figs. 10-15) confirming our previous data, have shown that the block-co-polymer CN-HA has the ability to increase the activity of CN or HA alone, further increased when it entraps other active ingredients as melatonin, vitamin E, betaglucan or lutein alone. This increasing activity on the synthesis of collagen and triple helix formation at the level of ECM supports and balances the activity of cytokines also (Figs. 10 and 11). Moreover CN-HA has shown an antioxidant activity (Fig. 13), further increased when entrapping known antioxidant ingredients such as vitamin C or melatonin. Finally it is interesting to underline the complete safeness of these block-copolymeric nanoparticles when controlled on keratinocyte and fibroblast cultures (Figs. 16 and 17).

As known while the different collagen molecules act as support of ECM, the protein HSP47 seems to be responsible of linking the different helixes, characterizing the alpha triple-helix formation of collagen [65-68].

As it's possible to see, the CN-HA block copolymeric nanoparticles show different effectiveness in relation to the entrapped active ingredients, so that it will be possible to select their use depending on the desired activity designed for the final cosmetic emulsion. Finally, the cytotoxycity (69) and the consequential tolerability, controlled on keratinocyte and fibroblast' cultures by the MTT method (MTT (3,(4,5-dimethylthiazol-2)2,5 difeniltetrazolium bromide) (37,65), are shown on (Figs. 16 and 17).

2.8 Step 6: Effectiveness of the Final Antiaging Formulation

The final control of the cosmetic formulations was verified by an *In vivo* study verifying the antiaging activity of the designed formulations by a multicenter randomized vehicle controlled study on 40 healthy women exhibiting sign of photoaging. The selected parameters i.e. skin hydration, TEWL, surface lipids and depigmenting activity, are shown in (Figs. 18-21) [65].

3. STATISTICAL ANALYSIS

Independent two-sample t-tests were used to compare treatment groups' demographic variable and outcome measures at baseline and each of the follow-up visits, and the change in measure from baseline to final visit. Categorial measures were compared between groups using X2 or Fisher exact test as appropriate. All results achieving two-tailed p value less than 0. 05 were considered statistically significant. Calculations were performed with SAS software, version 9.1(SAS Institute Inc, Cary, USA).

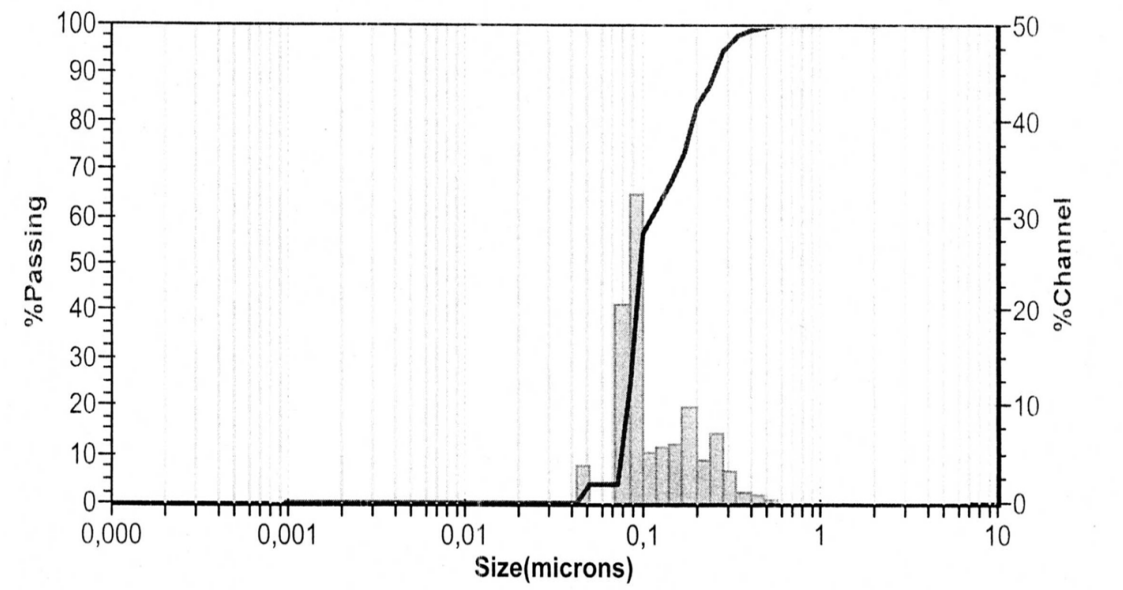

Fig. 9. CN-HA mean size physically controlled by the light scattering system and scanning electron microscope also

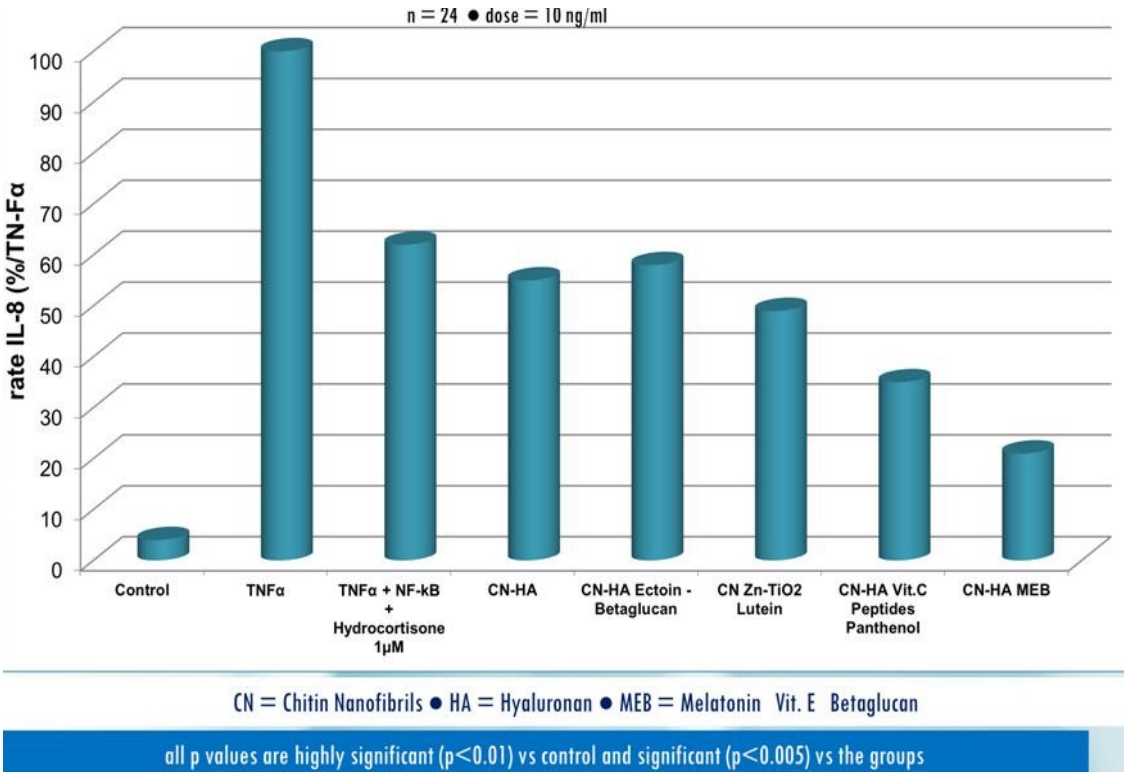

Fig. 10. Inhibition of IL-8 release on TNF-α stimulated human keratinocytes

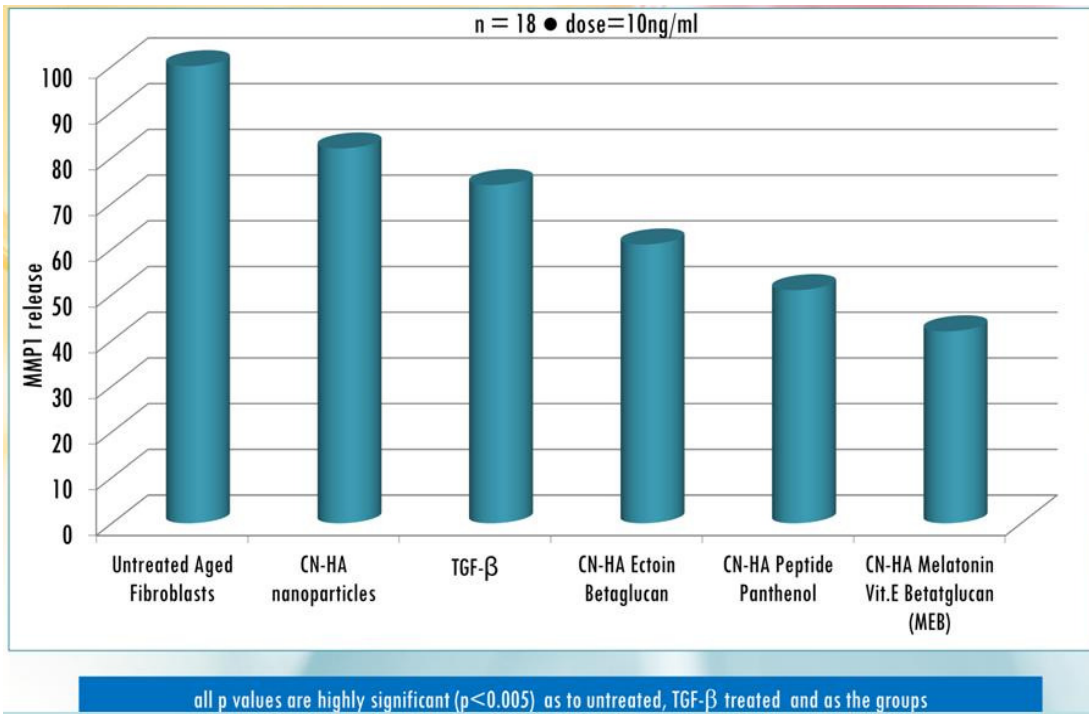

Fig. 11. MMP1 release in aged fibroblasts treated by chitin nanofibril-hyaluronan (CN-HA) entrapping active ingredients

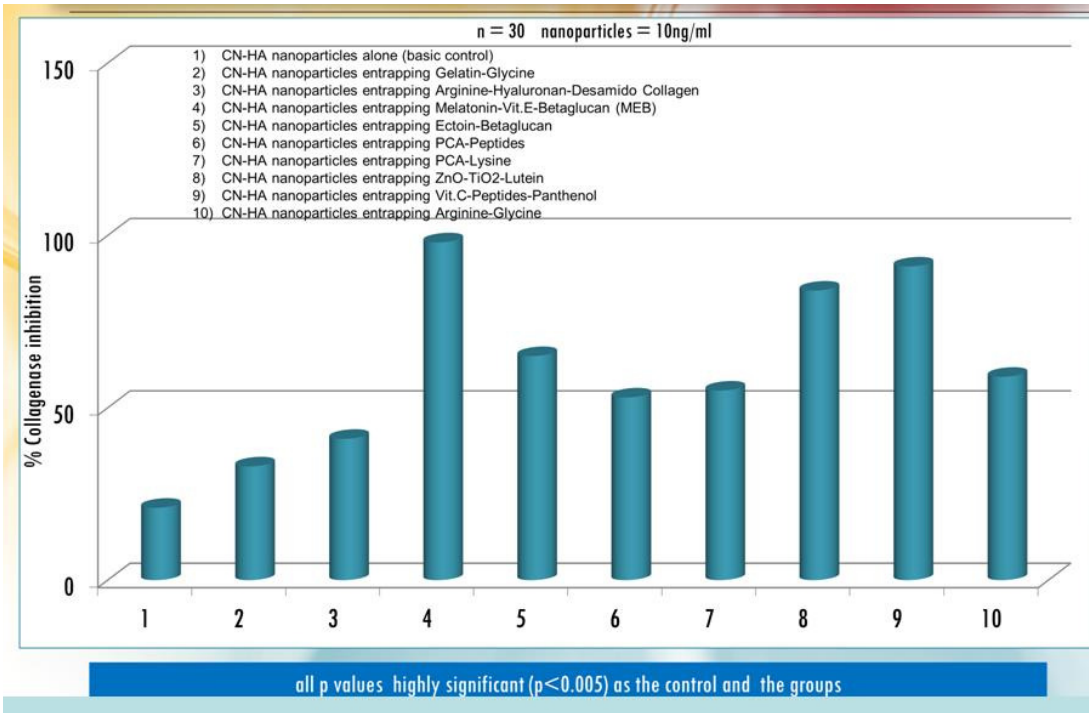

Fig. 12. Inhibitory activity of chitin nanofibrils-hyaluronan nanoparticles entrapping active ingredients in fibroblast culture incubated with collagenase enzyme vs collagen

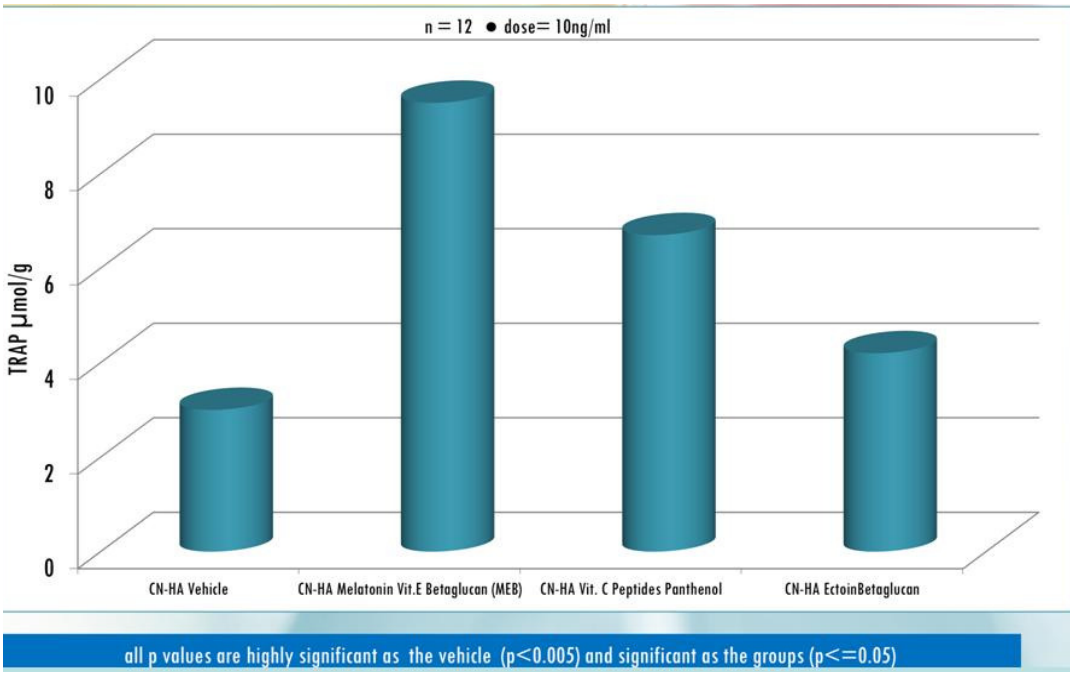

Fig. 13. Antioxidant capacity of chitin nanofibrils-hyaluronan nanoparticles (CN-HA) entrapping different active ingredients

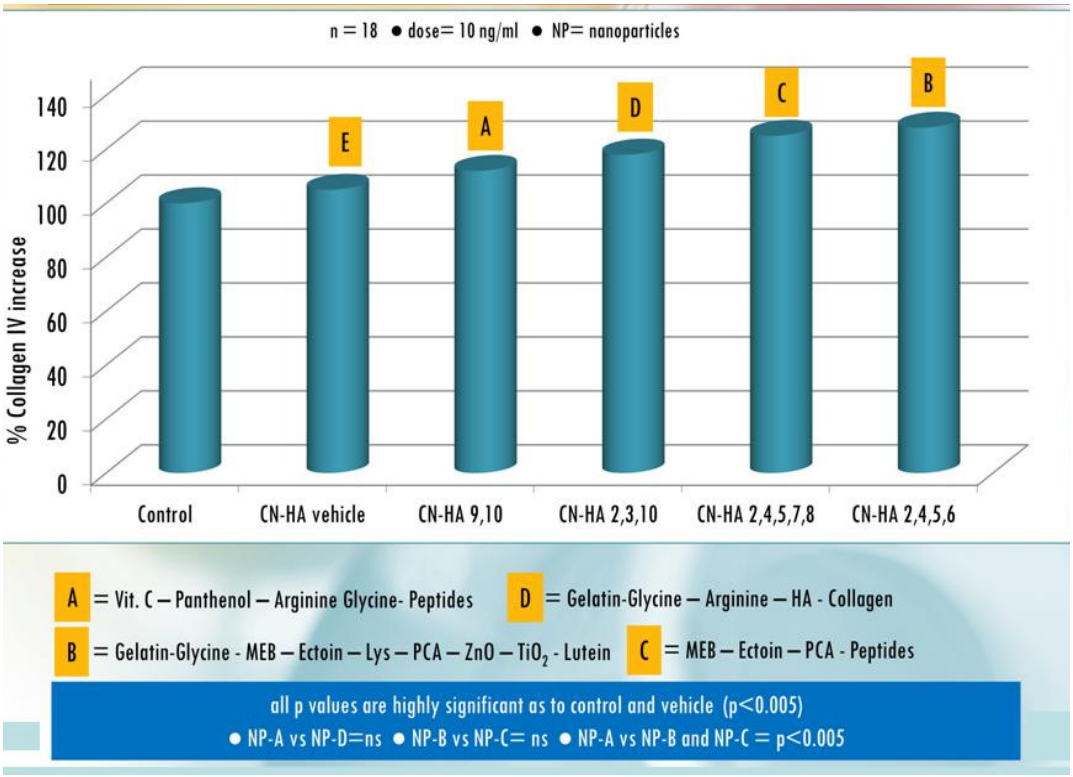

Fig. 14. Increase of collagen IV in fibroblast cultures added with chitin nanofibrils-hyaluronan (CN-HA) nanoparticles entrapping different active ingredients

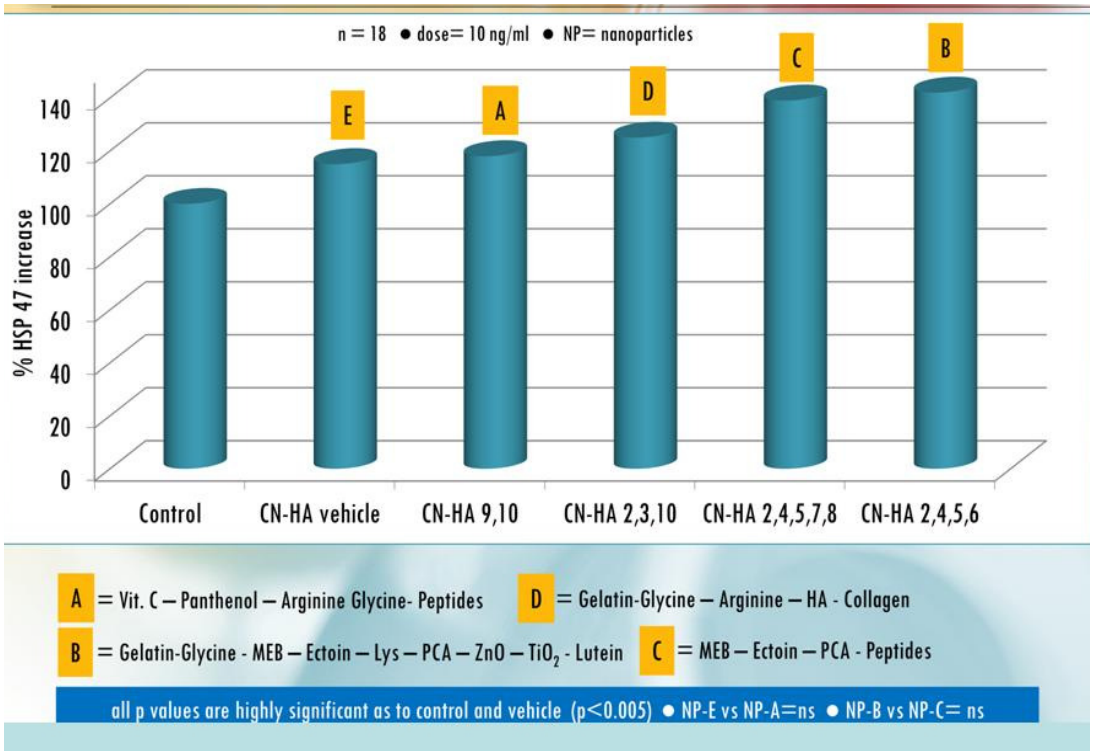

Fig. 15. Increase of HSP-47 in fibroblast cultures added with chitin nanofibrils-hyaluronan (CN-HA) nanoparticles entrapping different active ingredients

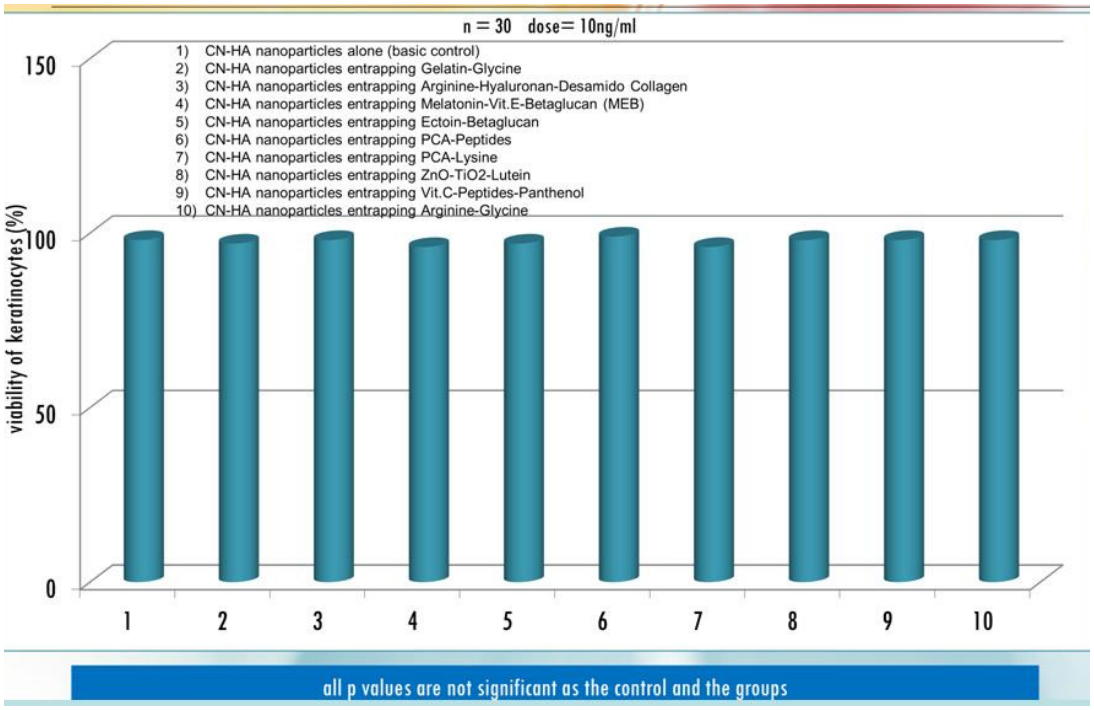

Fig. 16. Effect of chitin nanofibril-hyaluronan nanoparticles on the viability of keratinocytes

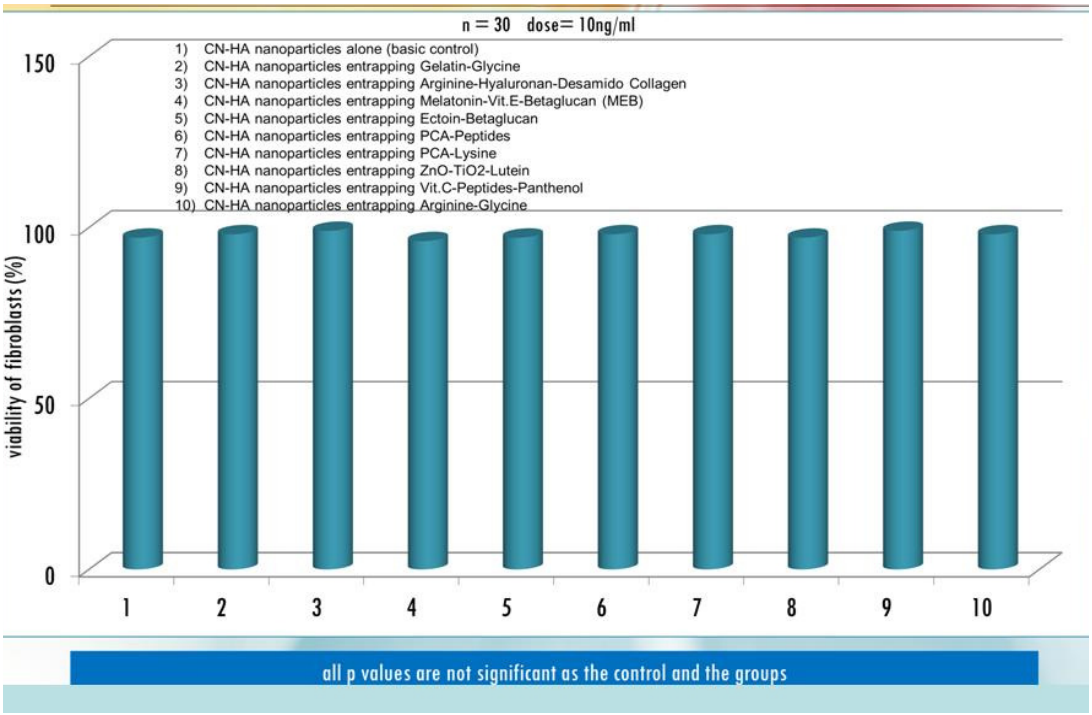

Fig. 17. Effect of chitin nanofibril-hyaluronan nanoparticles on the viability of fibroblasts

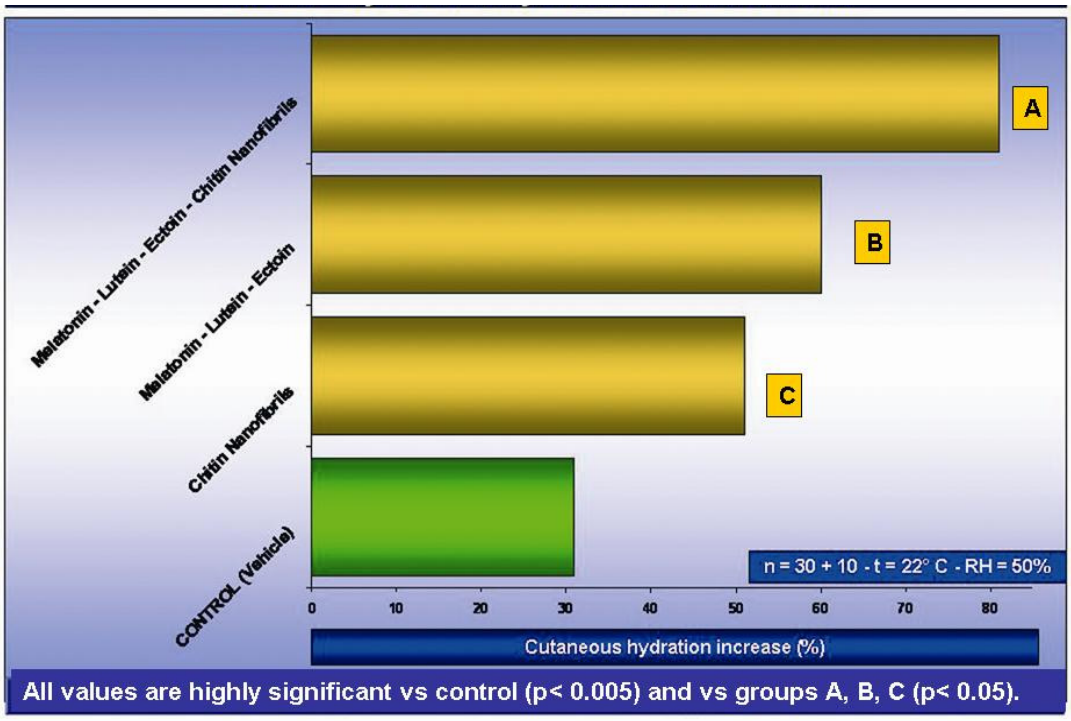

Fig. 18. Activity of chitin nanofibrils, antioxidant and immunomodulant compounds on skin hydration of women affected by dry skin after 60 days of bi-daily treatment on facial skin

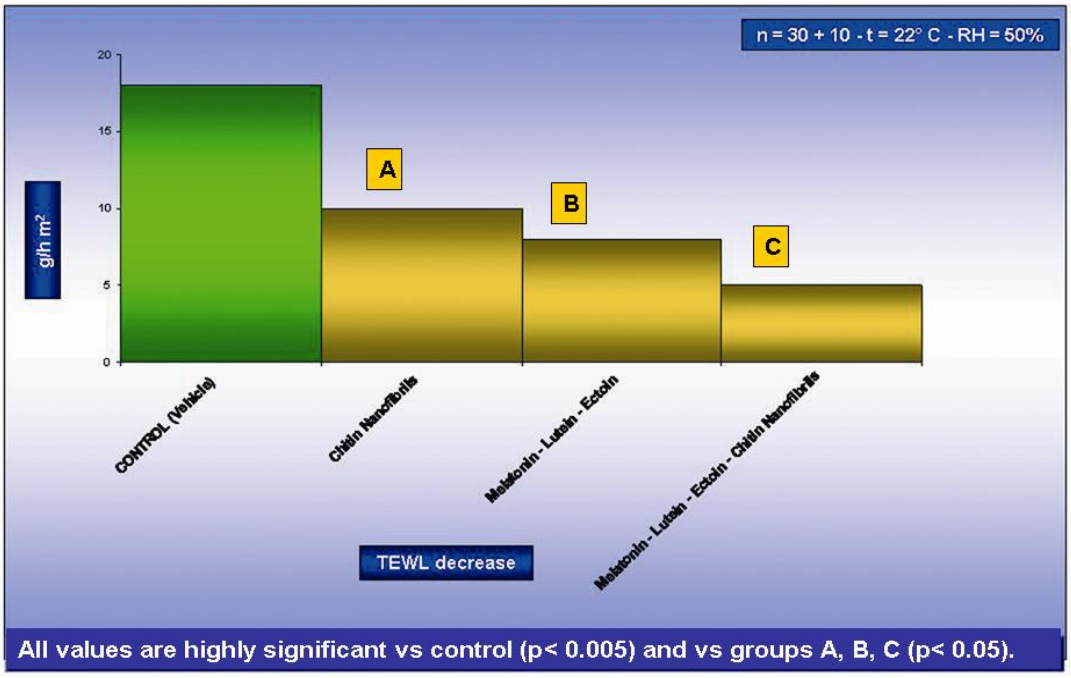

Fig. 19. Activity of chitin nanofibrils, antioxidant and immunomodulant compounds on TEWL recovered of women affected by dry skin after 60 days of bi-daily treatment on facial skin

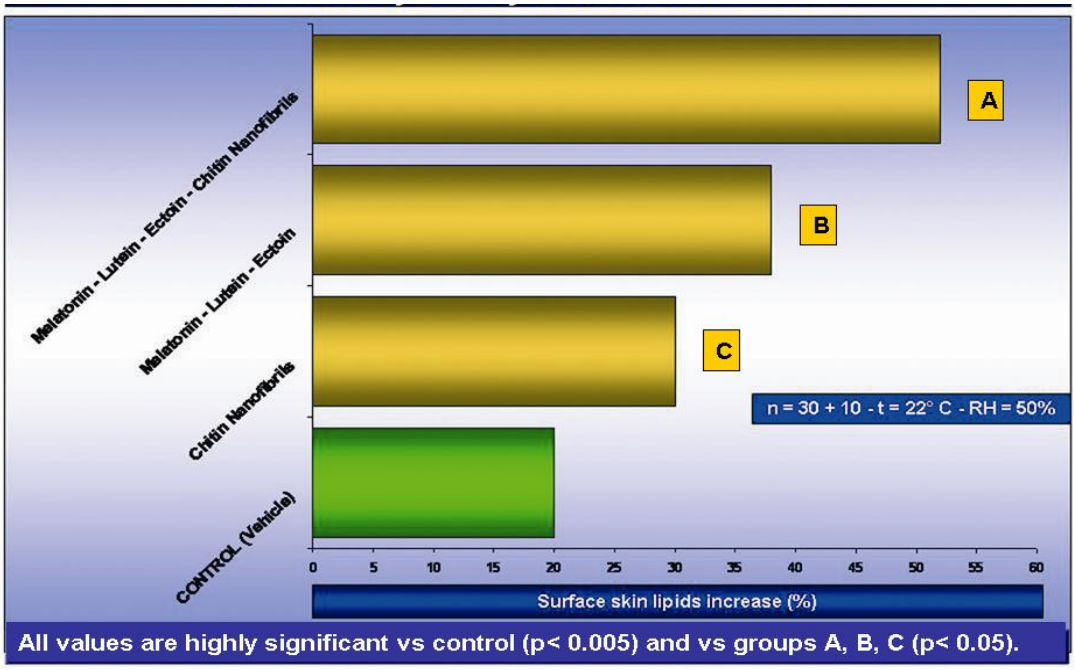

Fig. 20. Activity of chitin nanofibrils, antioxidant and immunomodulant compounds on surface skin lipidsof women affected by dry skin after 60 days of bi-daily treatment on facial skin

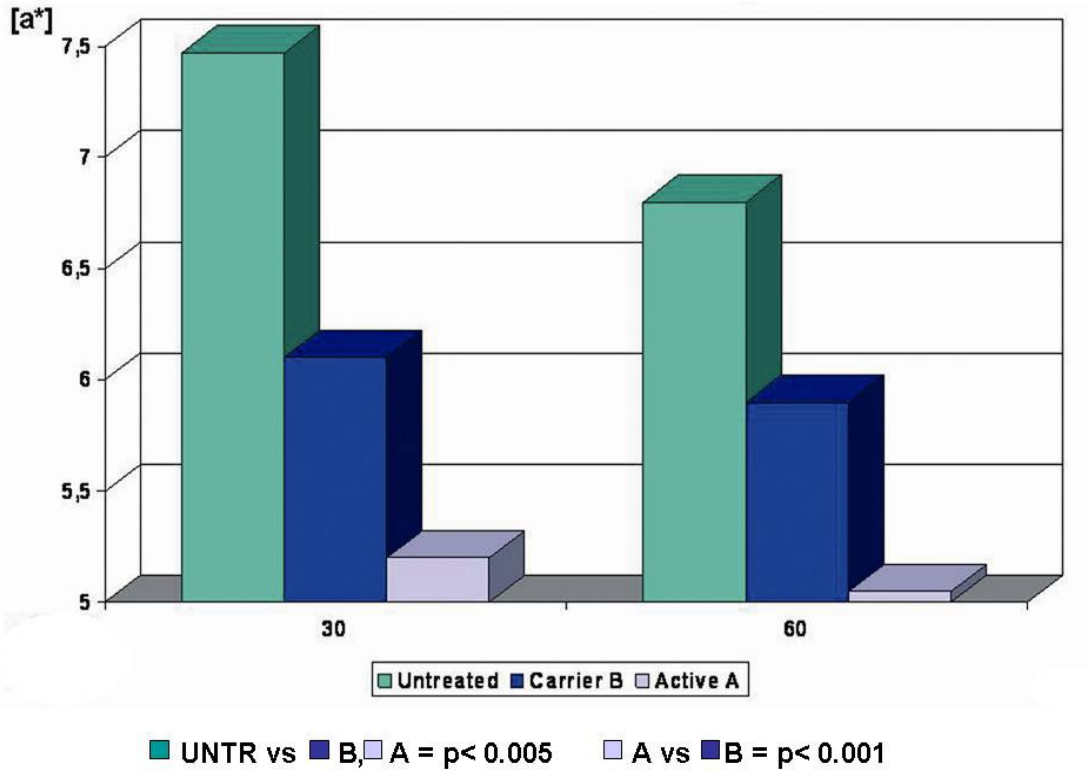

Fig. 21. Chromameter values a* (difference to baseline) of treated skin areas after 60 days application of a chitin nanofibrils gel

4. CONCLUSION

The human' Stratum Corneum (SC) is the uppermost layer of the epidermis consisting in flattened cells, the corneocytes, embedded in a mixture of lipids responsible for the skin' barrier function. These lipids, organized as lamellar layers, are considered to exist as bilayer or three layers sandwich-type structures, comprising of a softer, more fluid inner layer surrounded by outer layers of crystalline structure. In any way, organized as a plethora of linear and branched chains, and characterized by different physical and thermodynamic functions, they play a pivotal role in the barrier function contributing to rigidity and the tightly impermeable nature of the skin. Thus the SC structure constitutes the main barrier to penetration of exogenous substances through the skin. Therefore, the *first problem* for designing a cosmetic formulation to be distributed on the market place is to use safe raw materials and to solve the delivery of the active molecules into or via the skin, overcoming its barrier structure by the use of right vehicles. The solution could be to fluidize and temporary disorganize the SC-lipids, transforming the lipid crystalline-gel in the liquid crystalline-form, as happened by the use of this in-lab formulation. At this purpose, all the necessary controls are reported on Steps 1-5.

The CN-HA block copolymeric nanoparticles used and described seem to overcome this problem. They, when positively charged, generate interactions with the SC, whose overall net charges is negative. In fact, according to Shin et al. [70] results, the positive charges of CN-HA nanoparticles, dramatically increased the skin penetration of the active ingredients entrapped, overcoming the first problem of the skin barrier, as shown from our reported results [36-42].

The *second problem* is to select the right active ingredient (s), fundamental to obtain a positive reply from the skin' living cells, such as keratinocytes and fibroblasts, increasing their turnover and viability. In the area of skin care products there is, in fact, an increasing interest in effective ingredients possessing not only generic moisturizing properties, but having also effective

antioxidant and protective activities against the stress phenomena, associated with the formation or Reactive Oxygen and Nitrogen Species (ROS/RNS). The excessive presence of these free radicals at level of skin cells, propagating the chain reactions of lipid peroxidation, causes destruction of both the cell membrane and the ECM components, with a consequent skin aging. In photo aging the presence of the oxidizing free radicals is further increased for the combined activity of the UV rays. This is the reason for the use of the antioxidant compounds selected, whose activities was controlled on keratinocyte and fibroblast cultures. Keratinocytes, in fact, are the source of citokines and other peptides as *alert molecules* promoting inflammation, while fibroblasts are the machinery, producing collagen and other macromolecules. Thus, it was verified on keratinocytes the anti-inflammatory effectiveness of CN as raw material, while on fibroblasts it was controlled the anticollagenase and anti-metalloproteinase activities (Figs.10 and 11) of the emulsion, enriched with CNHA nano particles.

Some enzymes play, in fact, a major role in skin photoaging, showing proteolitic activity in degrading protein such as collagen and elastin [69-73].

As *third problem,* it is necessary to consider the macromolecules involved in living skin at level of derma-epidermis junction also. Skin wrinkles, in fact, are the result of continuous stresses and environmental aggressions causing not only cumulative skin inflammation and alteration of cell' cycles, but also destruction and lowering synthesis of filling macromolecules. In this case derma and dermo-epidermal junction, rich in macromolecules, become less dense and unable to adapt themselves to stress. This the reason to control the real influence on collagen synthesis of both active ingredients and the final emulsion. It is just the orchestra of these macromolecules that, globally involved in skin modifications of aging process, is of great interest in the area of skin care products.

It was controlled, in fact, not only the collagen synthesis as shown in (Fig. 12), but also the chaperon protein, involved in the triple helix structure, as reported in (Fig. 15), in order to underline the effectiveness of the active ingredients selected.

Being the final emulsions designed as antiaging cosmetic product, as *fourth problem* it seems interesting to underline another physiological process of human cell, the *glycosilation* that appears altered during the aging process. It is an enzymatic controlled process necessary to attach sugar moieties to protein molecules as reported on (Fig. 22).

At this purpose, Chitin Nanofibril (CN) could be considered a glycan compound capable to attach itself to the oxygen of collagen aminoacids, as hydroxyproline, or to act as glue for the SC' lipid lamellae, contributing to the physiological glycosilation process of the cell as well as to stabilize the structure of the lipid barrier. It is to remember that glycosilation is critical for a wide range of biological processes, including cell attachment to ECM and protein-ligand interactions in cell [74-76]. This means that CN-HA, as block copolymer of chitin and hyaluronic acid, could be used from the cell to support production of glycosaminoglycans also.

In conclusion, the reported results on the effectiveness and safeness of CN and CN-HA block copolymers as part of anti-aging micro/nano emulsions, overcoming all the described problems, open new challenges for designing innovative Clinically Correct Cosmetics. These so called *cosmeceuticals* could be used for skin minor diseases also, as reported by other considerations and studies published elsewhere from our research group [42,59,65].

Further studies are going on with the scope of utilizing CN-HA and other natural polymers to produce not only cosmetic and pharmaceutical emulsions, but also non-woven tissues for advanced medications and 100% biodegradable films for food packaging. These natural polymers positively charged, have shown to be safe compounds with a flexible activity, also because easily bonding with other natural polymers negatively charged. Last but not least, CN is a raw material obtainable from fishery's and crustaceans waste [16-20], easily forming block copolymeric nanoparticles with other natural polymer from plant biomass, such as lingo-cellulosic compounds [15], all abundant raw materials present worldwide as by-products, at quantity of billions tons/year at low cost [77].

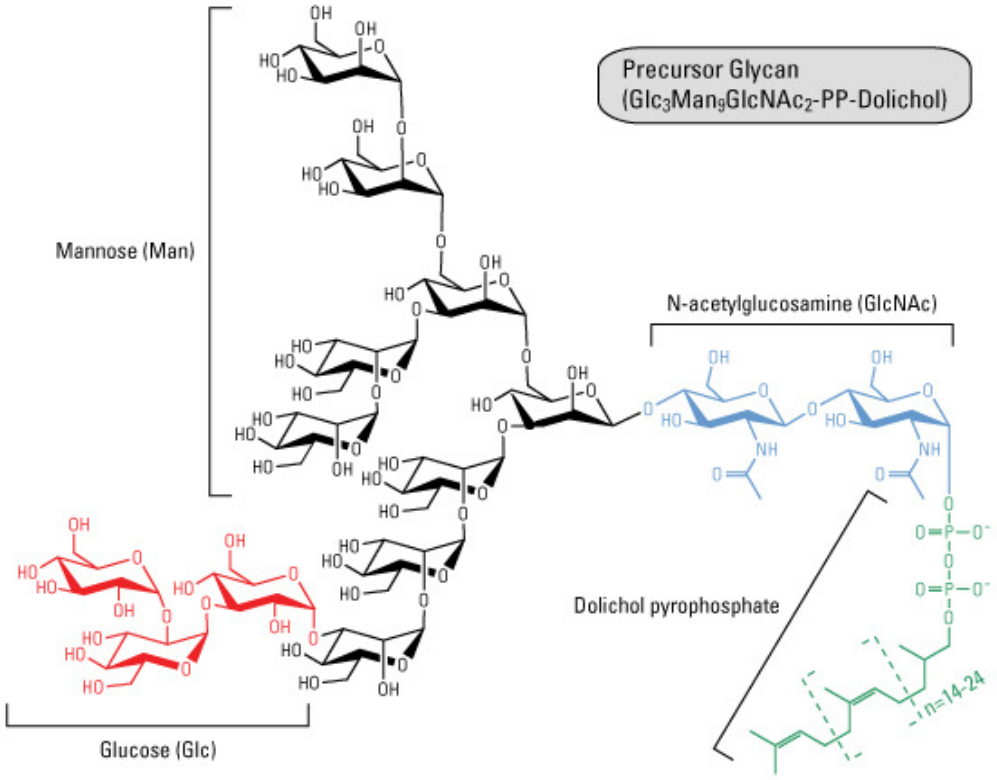

Fig. 22. Physiological process of glycosilation involving glucose and acetyl glucosamine

In conclusion it could be necessary to use this raw material by new nano-bio-technologies for bettering the managing innovation, lowering the costs, and reducing both pollution and green house gas emissions, to save the environment. This is the goal and the great future challenge of the so called *green economy* for a potential *zero waste* environment [78] with the capacity to address the biodiversity loss, which is already estimated to have a cost for Europe of about 450 billion €/year [79].

COMPETING INTERESTS

Author has declared that no competing interests exist.

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