HYALURONAN (HYALURONIC ACID) A NATURAL MOISTURIZER FOR SKIN CARE

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ABSTRACT

This chapter describes the chemical and physical properties of hyaluronic acid and its cleaved, lower-molecular-weight fragments as well as its derivatives relative to their interesting and useful topical application to moisturize skin in anti-aging products.

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4.1.3.1 STRUCTURE AND SELECTED PHYSICAL-CHEMICAL PROPERTIES OF HYALURONAN

Hyaluronan (HA) is a naturally occurring linear polysaccharide consisting of alternating β-1,4-linked units of β-1,3-linked glucuronic acid and *N*-acetyl-D-glucosamine (Figure 1.1). HA is a main component of the extracellular matrix in connective, epithelial, and neural tissues and is known to play an important role in the tissue hydration and water transport mainly due to its enormously high water-binding capacity. The high water-binding capacity of HA can be attributed to high density of fixed negative charges in HA chains from carboxyl groups, which causes the osmotic pressure and draws the water molecules into the tissues containing HA.[1] For example, HA within skin layer holds up 1000 times its weight in water molecules.[2] Additional biological functions of HA include maintenance and viscoelasticity of liquid connective tissues such as joint synovial fluid and eye vitreous, supramolecular assembly of proteoglycans in extracellular matrix, and numerous receptor-mediated roles in cells, mitosis, migration, tumor development, and metastasis and inflammation.[1]

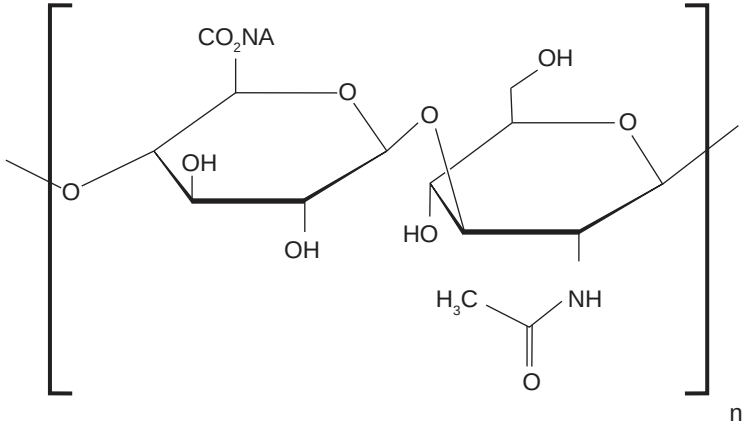

Figure 1.1 Hyaluronan (HA) structure.

The extensive repertoire of HA biological functions suggests the existence of different conformations and specific binding interactions.[3] It is likely that the conformation is affected by the local environment, including ionic strength and specific ion interactions, and the presence of interacting species including proteins and lipids. In the extracellular matrix, under normal physiological conditions and ionic strength, HA is generally believed to exist as a crowded random coil molecule.[4] A change in HA conformation is suspected under specific pathological conditions. Lowering the pH results in protonation of carboxyl groups and increased solution viscosity. In contrary, there is a polyanion contraction with rising ionic strength

due to the increased electrostatic shielding of HA. This latter effect is accompanied by a decreased solution viscosity. The HA solution viscosity is also temperature dependent and is a function of the overlapping parameter, molecular weight and concentration.

Some applications involving HA as an active ingredient require filtered HA solutions. The question that arises is whether filtration changes the physical-chemical properties of HA or not. According to our unpublished results, when proper dilution is used, filtration of HA (from 15 kDa up to 1.7 MDa) solutions through 0.22 μm nylon filter had no impact on molecular weight, polydispersity, and solution viscosity. The filtration impact on biological properties has not yet been established.

The major disadvantage of native HA is its low resistance against enzymatic degradation and short half-life after internal administration due to natural scission by enzymes and degradation by radicals. For this reason, at the present time the main challenge is the design of new HA materials that retain or increase the hydration properties of the polysaccharide and at the same time last longer in the application point. In order to increase the biological resistance of HA towards enzymatic degradation, modifications are being introduced in HA structure.[5,6] Another challenge is to synthesize HA derivatives with altered and specific physical-chemical and biological functions. Since the biological properties are connected with the HA molecular weight, there is also a great interest in HA degradation and evaluation of biological behavior of HA fragments.

4.1.3.2 PREPARATION OF HYALURONAN FRAGMENTS, ISOLATION AND CHARACTERIZATION THEREOF, CHARACTERIZATION OF DEGRADATION PRODUCTS

Disintegration of the HA β-linkages leads to formation of hyaluronan fragments of different sizes, identified by a name that indicates the number of monosaccharide

units of which they are composed, e.g., a tetramer consists of four monosaccharide units—two glucuronic acids and two *N*-acetyl-D-glucosamines (GlcNAc). The name of the other fragments is created analogically. Fragments comprising more units are characterized by average molecular weight in Daltons. Thus, an eicosamer having 20 subunits is in literature specified as 20-mer, but it is also possible to say HA of molecular weight 4 kDa.

Mechanisms of the HA cleavage into its smaller fragments comprise enzymatic, free radical, thermal, ultrasonic, and chemical methods such as acid and alkaline hydrolysis.[7] Non-enzymatic reactions proceed randomly, and by this means mixtures of HA saccharides with different molecular weight can be obtained. By contrast, enzymatic cleavage is selective and allows preparation of HA

oligosaccharides with specific structure. Enzymatic digestion is catalyzed by two main types of enzymes—hyaluronate lyase and bovine testicular hyaluronidase (BTH).[8] Both enzymes produce fragments that contain an even number of saccharide units with GlcNAc at the reducing end. While HA oligosaccharides obtained by BTH are fully saturated, hyaluronate lyase produces oligosaccharides with the double bond between C-4 and C-5 position of uronic acid at their nonreducing end. Both saturated and unsaturated HA oligosaccharides lose under alkaline conditions GlcNAc from the reducing terminus with formation of odd-numbered oligosaccharides.[9]

During non-enzymatic cleavage, HA can be partially transformed into undesired degradation by-products exhibiting unwanted biological properties like carcinogenesis. Their identification is important so as to suggest ways of their elimination and to offer a final fragmented HA product free of toxic impurities. Until now only degradation by-products arising from acidic and alkaline hydrolysis have been established. Cleavage of hyaluronan under alkaline conditions gives a mixture of stereoisomers (*E*)-3-dehydroxy-3-en-D-GlcNAc and (*E*)-3-dehydroxy-3-en-D-ManNAc,[8] while acid hydrolysis of hyaluronan leads to creation of furan-like derivatives and derivatives of cyclopentanone.[10] These degradation by-products can be removed from the reaction mixture by ultrafiltration through a membrane of suitable cut-off.

Physical-chemical properties and biological activity of native HA significantly differ from its low-molecular-weight fragments. While high-molecular-weight HA is a space-occupying material and contributes to the structure of the extracellular matrix and tissue integrity, smaller-sized HA fragments are involved in a wide spectrum of complex biological mechanisms. From the cosmetic perspective, the most important properties of HA fragments involve stimulation of angiogenesis and supporting the proliferation of fibroblasts. Pro-angiogenic properties are

exhibited especially by short HA oligosaccharides comprising from 6 to 20 saccharide units. HA oligosaccharides are mitogenic for endothelial cells, support their migration, and induce multiple signaling pathways.[11] Their good biocompatibility and low toxicity make them an ideal therapeutic agent for the situations when stimulation of new blood vessel growth is needed. *HA oligosaccharides are also components of dermatological compositions for combating hair loss and stimulating hair growth*. In the field of reproductive medicine, HA oligosaccharides may help fertilization.

Hyaluronan fragments can also be used for treating and preventing wrinkles, expression lines, fibroblastic depletion, and scars. HA oligosaccharides (with molecular weight <5 kDa) contribute to the suppression of wrinkles in both layers of the skin. At the epidermis, they stimulate the endogenous synthesis of highmolecular HA, which has a positive effect on hydration. At the level of the dermis,

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HA fragments decrease the creation of pro-inflammatory interleukins, which are responsible for generation of free radicals capable of damaging both components of the extracellular matrix and the skin cells themselves. The most ambitious application of HA oligosaccharides is treatment of cancer. Anti-tumorigenic effects of HA oligosaccharides are based on their ability to sensitize cancer cells to treatment. The binding of oligomers to the HA receptors also inhibits the pro-tumorigenic signaling of high-molecular-size HA. Some low-molecular-weight HA molecules are believed to release strong immune reaction to the cancer cell.[12-14]

Analytical Techniques for Characterizing HA Molecular Weight

Since the length of HA polysaccharide chain is closely related to its biological functions, it is necessary to determine its molecular weight properly. A range of analytical techniques has been employed to assess the sizes of HA fragments. These were originally determined from the ratio of total glucuronic acid concentration to that of the reducing end GlcNAc group. More recently, other methods including viscosimetry, electrophoresis, mass spectrometry, and liquid chromatography have been used.[15] Electrospray ion mass spectrometry (ESI-MS) is mainly used for molecular weight characterization of short HA oligomers (up to 40-mer) derived from enzymatic digestions.[16] Enzymatic degradation provides a complex mixture of even-numbered HA oligomers, which must be fractionated using suitable techniques such as electrophoresis or liquid chromatography before ionization. ESIMS utilizes a flowing stream containing the analyte and provides gentle ionization. Proper care must be taken in setting the cone voltage that accelerates the ions into the mass analyzer because high values of the cone voltage

may result in an artifact of an increase of charged species. By contrast, low cone voltage leads to insufficient ions. At lower cone voltage it is possible to observe odd-numbered HA oligomers as artifacts in ionization.

As in the ESI-MS technique, it is not possible to avoid fragmentation of larger HA oligomers; it is advisable to use MALDI-TOF. This technique is suitable for characterization of polydisperse mixtures; it presents tolerance towards salts and buffers and is highly sensitive.[17] However, MALDI-TOF cannot be considered a quantitative method because desorption of HA fragments differs according to their size extensively. These complications can be overcome by using size-exclusion chromatography (SEC) coupled with multi-angle light scattering detector (MALS) and refractive index (RI), which provides detailed information of molecular weight, molecular weight distribution, molecular size, and solution properties in a wide range of HA fragments.[18] A limitation of the technique is that an SEC column cannot separate HA fragments sufficiently. Hence, two SEC analytical columns packed with an appropriate resin and connected in series are the possible solution for the separation of HA fragments. In this set, the length of the analysis is still acceptable.

4.1.3.3 PREPARATION OF CHEMICAL DERIVATIVES OF HYALURONAN AND THEIR CHARACTERIZATION

As it has been mentioned earlier, native HA suffers from its low resistance towards enzymatic digestion. This means that native HA has a high rate of *in vivo* turnover, which limits the period of time during which HA may act in the place of application. For this and other reasons, chemical modifications of HA intended for cosmetic or pharmaceutical applications are recommended. Chemically modified HA derivatives are also usually more mechanically robust and thus they are more suitable for final products manufactured using higher temperatures, pressures, or shear stress. *Modified HA derivatives usually have stronger binding capacity to the skin*, and as a result they are not washed off as easily as unmodified HA. An extensive research has been developed for chemical modification of HA. The strategies employed can be divided into two categories. The first one involves chemical attachment of pendant groups to change the properties of the polysaccharide and modulate the hydrophilic character of HA. The usual aim of these modifications is to lower the hydrophilicity of HA, so that the modified biopolymer will be able to blend with hydrophobic materials typically used in cosmetic products.

The second strategy for chemical modification of HA involves cross-linking in order to prepare insoluble derivatives of HA or hydrogels.[19] In fact, almost all of

the commercially available HA products are stabilized by cross-linking to obtain materials with longer residence times and increased mechanical robustness.

Regardless of the modification strategy, the new, modified HA materials should remain biocompatible and biodegradable, as well as retaining the moisturizing properties. A critical point in the synthesis of HA derivatives is the preservation of the original chain length, since HA lubricant, shock-absorbent, and space-filling properties are closely correlated to its high viscosity, which is directly related to the initially high molecular weight of the biopolymer. From our personal experience, degradation is always inevitable during chemical modification processes.

Chemical modification of HA

Such modification can be carried out by the addition of the following functional groups to the polysaccharide backbone: carboxyl, primary or secondary hydroxyl groups, or the amino group after deacetylation. The chemical modification of carboxyl group is often preferred because it is the recognition site for HA natural receptors and hyaluronidase.[20] This method of modification may decrease the HA susceptibility towards enzymatic degradation.[21] However, the biological behavior of the polysaccharide, including its biodegradability, may change after such derivatization. Thus, the effect of each modification on the biocompatibility of the polysaccharide must be investigated in order to characterize the result on its properties.

HA coupling via its carboxyl group requires its previous activation to make it reactive to nucleophiles. The modification of carboxyl groups produces new amide or ester bonds in HA backbone. The typical reagents are the same as those used in common peptide synthesis, such as water-soluble carbodiimides (EDC and derivatives), carbonyldiimidazole (CDI), carbonyltriazole (CDT), N-hydroxysuccinimide (NHS), *p*-nitro-phenol, p-nitrophenyltrifluoracetate, and ethyl chloroformate (ECF). Chemical modification of HA is primarily performed in water or in organic solvent. To carry out modification in organic solvents such as DMSO, DMF, or formamide, a previous conversion of sodium hyaluronate to its acid form has to be done. However, this conversion drastically degrades the polysaccharide. Thus, the modification in water is always preferred. Previously enounced methodologies are in general patented and very well known by people skilled in the art. These strategies have been used for the synthesis of nano-emulsifiers,[22] films,[23] hydrophobized HA,[24] or hydrogels.[25]

On the other hand, the modification of the hydroxyl groups may involve the formation of ether bonds by reaction with bis-epoxides.[26] An example is the commercially available **Juvederm®**, a commonly used filler in cosmetic surgery. This product is chemically cross-linked by divinyl sulfone. Hydrophobized HA can be synthesized via formation of carbamoyl bonds after activation of hydroxyl groups.[27] The oxidation of the primary hydroxyl has been used for bioconjugation with lipids and subsequent hydrophobization.[28] However, some allergic reactions have been recently reported for commercially available products,[29] and so new challenges have appeared for scientists devoted to HA chemistry.

In the case of the formation of insoluble derivatives of HA (hydrogels), the capacity to retain water by the polysaccharide should be always determined by measuring the degree of swelling. *The denser the hydrogel is, the lower its water uptake capacity and swelling ratio.* In fact, regarding HA, most of the experimental data indicate that high swelling ratio values correspond to the lowest modification of the biopolymer. Low degree of substitution usually results in the formation of hydrogels with low cross-linking density, resulting in larger absorption of water and thus high swelling ratio. Conversely, high degree of substitution helps to form homogenous and more compact hydrogel network with lower water uptake capacity. Compression tests are often used to determine the smoothness of the hydrogel. Additionally, rheological studies are essential to evaluate the effect of derivatization or crosslinking.[30] Typically, the rheological behavior of HA and its derivatives is characterized by non-Newtonian behavior. The mechanical integrity of HA hydrogels is usually determined by measuring shear modulus (G), the larger the G value, the more resistant network towards deformation. The G value usually increases with increasing HA modification. After chemical modification it is always necessary to evaluate the effects of the derivatization on the physical, chemical,

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and biological properties of the polysaccharide, as well as possible degradation during the process.[31]

4.1.3.4 HYALURONAN PENETRATION INTO THE STRATUM CORNEUM AND INTO THE SKIN

Skin is the largest reservoir of HA in our body, since it accounts for 50% of the total body HA content. Under ideal conditions, HA is found in all the layers of the epidermis and dermis. In the epidermis, HA is most prominent in the upper spinous and granular layers, where most of it is extracellular. By contrast, the basal layer of the skin also contains HA but it is predominantly intracellular. Presumably, basal keratinocyte HA is involved in cell-cycling events, whereas secreted HA in the upper outer layers of epidermis is part of the mechanism for dissociation and

eventual sloughing of cells. The HA content in the dermis is far greater than that of the epidermis, and this accounts for most of the HA content in skin. The HA of the dermis is in equilibrium with the lymphatic and vascular system, while epidermal HA is not.[2]

Both epidermal and dermal cells are able to synthesize HA throughout our lifetime. However, *the skin cells lose their ability to produce optimal amounts of HA during the aging process. That is why the skin of babies is very rich in HA and is so soft, full, smooth, and supple, while with increasing age dehydration occurs; the skin thins and wrinkles appear.* The decline in HA production is also accompanied by a decreased suppleness, reduced elasticity, and loss of skin tone. The most dramatic histochemical change observed in senescent skin is the marked decrease in epidermal HA. In senile skin, HA is still present in dermis, whereas the HA of the epidermis has disappeared entirely. In fact, recent studies have indicated that the total level of HA in dermis remains constant with aging. However, at the same time, the proportion of total glycosaminoglycan synthesis devoted to HA is greater in the epidermis than in the dermis, and the reasons for precipitous fall with aging is unknown at the present time.

In order to retain the aesthetic appearance of the skin, and treat the signs of "dermatological" aging, it is recommended to keep "refilling" skin with HA from adolescent age onwards. It is already known that HA taken orally does not show any benefit to skin, because skin cells are not able to extract HA from the bloodstream.

The more reliable HA skin refill is by applying HA topically. Topical application of high-molecular-weight native HA (>500 kDa) is not recommended, because it does not efficiently penetrate the deeper skin layers mainly due to its large size. Instead it forms films that act as barriers against moisture loss.

4.1.3.5 MOISTURIZING PROPERTIES OF NATIVE HIGH-MOLECULAR-WEIGHT HYALURONAN

Conversely, HA fragments sized smaller than 500 kDa are claimed to permeate and moisturize the skin. An efficient uptake of HA (357 kDa) by epidermis is indicated in Figure 4.1, where an isotopically labeled ^{14}C-HA was incubated with full thickness porcine ear skin using Franz diffusion cells at 37°C for 48 hours. *However, at the same time there was hardly any HA uptake deeper into dermis layers.* This *in vitro* penetration test into the porcine skin should resemble what will, with high probability, occur *in vivo* in humans. The results suggest that HA of 357 kDa applied topically is useful for the replenishment of HA levels in epidermis but is still too large to efficiently penetrate into dermis. Smaller HA fragments are then needed for more efficient penetration when dermis targeting is required.

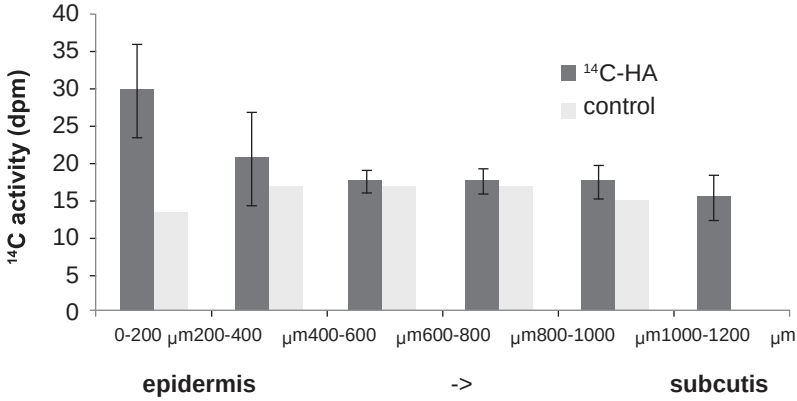


Figure 4.1 Penetration of HA Fragments: ^{14}C-HA activities within 200 µm skin slices (pigs ear) after 48 hours penetration at 37°C.

4.1.3.5 MOISTURIZING PROPERTIES OF NATIVE HIGH-MOLECULAR-WEIGHT HYALURONAN AND HOW THE MOISTURIZING WEIGHT IS REDUCED PROPERTIES OF HYALURONAN CHANGE AS THE MOLECULAR

Due to the unique hydration, viscoelastic, and biocompatibility properties of HA and in view of a range of molecular-weight materials' ability to penetrate skin, *HA is being extensively used in cosmetic products as moisturizer, thickener, and stabilizer*. Since HA is a water-soluble product, it can be easily incorporated in the water phase of cosmetic formulas.

Due to its negative charge, HA is incompatible with cationic compounds or proteins at certain concentrations. A typical concentration of HA in cosmetic products varies between 0.01 and 0.2%[32]. *HA is suitable for application in a wide range of cosmetic compositions devoted to skin care and designed for topical applications.* Typical cosmetic products supplemented with HA molecules include serum,

moisturizers, creams, shampoos, conditioners, and bath oils. The benefits of HA being in the formulation are usually connected with reduction of the dermatological aging, reduction of the depth of lines and wrinkles (including fine lines), decrease of skin fragility, amelioration of skin atrophy, improvement of skin firmness and texture, decrease in pore size, restoration of skin brightening, and improvement of skin barrier function.

In hair-care and hair-treatment formulations, HA restructures the keratin fibers "inside" the fibers, improves elasticity of hair, and increases the washing resistance of colored hair.

The advantage of HA over other widely used moisturizers (glycerin, propylene glycol, and sorbital, polyethylene glycol) resides in the fact that its moisturizing properties are not significantly influenced by relative humidity. Unlike the other moisturizers, HA is able to keep good water retention capacity both at low and high relative humidity. In contrast, common moisturizers at low humidity and/or under xerotic conditions (dry skin, deep wrinkles, and squamous skin) absorb moisture from the inner skin rather than from the air, which in turn causes the skin to dry out. Together with other substances, HA belongs to Natural Moisturizing Factor (NMF) substances, which are hygroscopic, act as a surfactant, and buffer the skin thereby preserving its aesthetic appearance.

The moisturizing properties of HA are related to its hydration, often referred to as an average number of water molecules affected by the presence of HA anion in aqueous solutions. HA hydration was studied using several approaches including NMR,[33] viscosimetry, ultrasonic velocimetry, and thermal analyses.[34] Among these, thermal analyses, namely differential scanning calorimetry (DSC), is able to estimate i) non-freezable bound, ii) freezable bound, and iii) free water due to their different thermodynamic properties caused by the presence of HA (Figure 5.1). The non-freezable bound water is the fraction of total water content that is in close contact with HA surface and it is supposed to be either hydrogen bonded to HA molecules or is restricted by junction zones formed by HA chains. The freezable bound water is the next layer, which interacts weakly with HA molecules and non-freezable water layer. Free water is not influenced by HA and its properties resemble pure water. Therefore, it is mainly the amount of non-freezable and freezable bound water, which is considered as the number of water molecules hydrating HA structure. The number of bound non-freezable water molecules in 650 kDa HA is 7.6 per disaccharide HA unit. In terms of freezable bound water, the hydration increases up to 43 molecules per HA disaccharide unit.[35] In terms of grams, this means that 1g of 650 kDa HA can strongly hold from 0.8 to 2g of water. Further DSC results indicate that the water-binding capacity is independent of molecular weight from 30 kDa up to 750 kDa. Increased hydration (for about 0.12 g of water per g of HA) was detected for 1.4 MDa HA.[36]

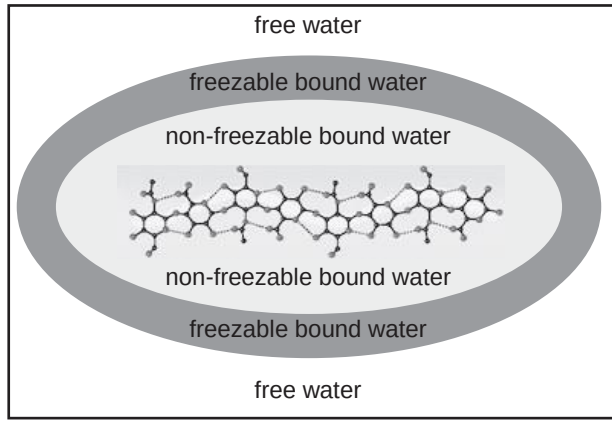
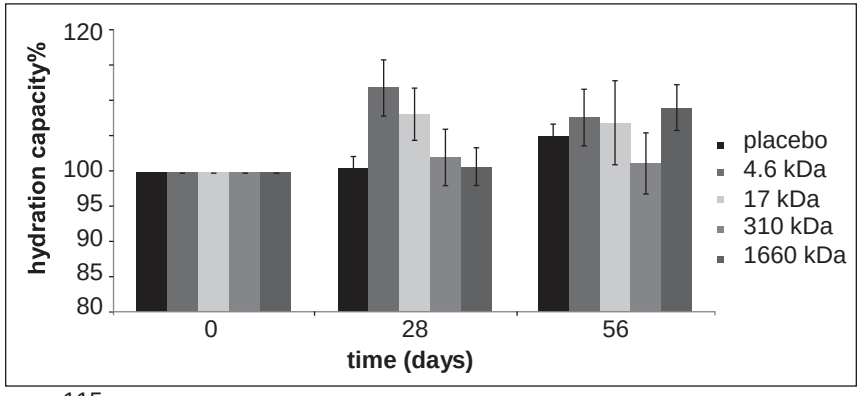


Figure 5.1 Depiction of freezable-bound, non-freezable-bound and free water molecules in the presence of HA

To estimate the effect of different molecular-weight HA on skin hydration properties, a group of volunteers (n = 8) applied for 60 days (1 time per day) an o/w emulsion containing 0.005% native or fragmented HA. The hydration results reported in Figure 5.2 indicate that in the case of smaller HA fragments, namely 4.6 and 17 kDa HA, there is a significant skin hydration capacity increase after the first month of emulsion application. This result is in agreement with easier and faster penetration of smaller-sized molecules into the skin and with the fact that at epidermis small HA fragments stimulate the endogenous synthesis of high-molecular HA and in this way positively affect skin hydration properties. However, the more efficient hydration tendency of small-sized HA fragments was completely suppressed after another two months of emulsion application, where the best results were obtained for the native 1.66 MDa HA. As it was discussed above, the high-molecular-weight HA did not penetrate the skin but formed an efficient barrier against moisture loss. The results of the three-month study indicate that the skin hydration capacity is both HA molecular size and application time dependent.



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110
105

Figure 5.2. Changes in hydration capacity of skin after the treatment with 0.005% HA in o/w emulsion.

4.1.3.6 SELECTED DERIVATIVES OF HYALURONAN AND THEIR EFFECT ON SKIN MOISTURIZING

Although a great number of HA derivatives have been synthesized in the last 15 years, there is a scarcity of literature devoted to the impact of HA modification on its moisturizing properties.

In general, it is believed that increasing the hydrophobic character of HA will enhance its penetration through the hydrophobic stratum corneum into the epidermis. Then, due to the fact that the permeability of substances through skin is not only related to their lipophilic character but is also inversely related to their size, small-sized hydrophobic HA fragments should theoretically favor transdermal transport.

To approve or disapprove the theoretical prediction, hydrophobized HA (50 kDa HA modified with 50% of hexyl functional groups) was tested *in vivo* for moisturizing effect. Similarly as in previous case, hydrophobized HA was integrated in o/w emulsion, which was daily applied by a group of eight volunteers. The changes in skin hydration capacity were compared to other two groups of volunteers using placebo and 17 kDa HA (Figure 6.1). In this case, the results indicated that the hydrophobized HA enhanced skin hydration capacity comparably to unmodified HA fragment. However, the effect on skin-moisturizing properties of tailor-made hydrophobic HA derivatives is unknown and still remains to be elucidated.

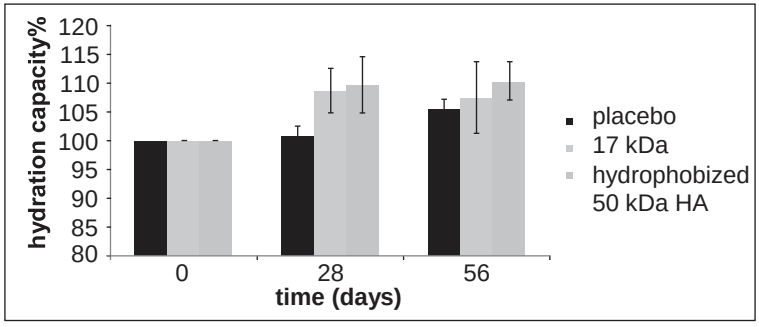

Figure 6.1. Changes in hydration capacity of skin after the treatment with 0.005% unmodified and hydrophobized HA in o/w emulsion.

Brown and colleagues[37] found that topical unmodified HA significantly enhanced the partitioning of both diclofenac and ibuprofen into human skin when compared to an aqueous control, pectin, and carboxymethylcellulose. This suggests

that glycosaminoglycans, when allowed to interact with water, can enhance the percutaneous penetration of some drugs and cosmeceuticals useful in cosmetics and personal care. The details of their interaction remain to be elucidated.

In this matter, hydrophobized HA may represent even more efficient transdermal drug delivery systems because of two main reasons. First, as it was mentioned earlier, the partial hydrophobicity should increase the permeation rate of HA through the stratum corneum. Second, the hydrophobized HA contains both hydrophilic and hydrophobic functional groups, which under certain conditions may enable formation of micro and nano vehicles. These are able to increase the stability of poorly soluble lipophilic components and nutrients. The transformation of HA molecules into liposomes, microemulsions, or polymeric micelles can be used in transdermal research as a better alternative method to enhance permeation of components through skin. In fact, penetration of small hydrophobic drugs from such delivery systems into the epidermis can be increased more than two times.[38] Unlike other polymeric based delivery systems, HA-based polymeric micro and nano structures have major advantages due to excellent biocompatibility and biodegradability of the backbone. Thus, the feasibility of native HA and its derivatives for the development of new materials for applications in cosmetic, biomedical, and pharmaceutical fields is still under review.

4.1.3.7 COSMETIC APPLICATIONS FOR VARIOUS MOLECULAR WEIGHTS OF HYALURONAN

The skin hydration process proceeds through a very complex mechanism. Regarding HA, the whole range of molecular weights of HA is related to skin moisturizing. However, as it was described in details above, the exact moisturizing mechanism differs with HA molecular weight. For this reason, the choice of HA fragment for cosmetic composition should be always considered in context with intended reason of use and application. Simplifying the problem of Mw choice, the larger the Mw of hyaluronan the more predominant are physical-chemical properties, while biological properties will prevail in case of smaller Mw fragments.

For this reason, high-molecular-weight HA (about 1 MDa) is usually added in cosmetic formulation in order to increase composition viscosity and improve the stability of composition film when applied on skin. In this way, high-molecularweight HA has positive effect on hydration of upper epidermis layers, which is manifested by lower trans-epidermal water loss (TEWL). Penetration properties (and thus anti-aging effect) of high-molecular-weight HA can be improved by combination of HA with skin penetration enhancers.

HA with molecular weights around 250 kDa enables deeper skin hydration due to its deeper penetration, where HA interacts with skin cells and extracellular matrix components. It is efficient in skin texture enhancement and wrinkles reduction.

Very small HA fragments (50 kDa and below) are very useful in anti-aging because after their penetration into deeper skin layers they serve as signal molecules for skin cells to synthetize new HA molecules. In addition, small HA fragments are

easily hydrophobized and in this way they penetrate deeper into epidermis and dermis, enhancing skin hydration capacity and enabling deep transdermal transport of complexed or bound hydrophobic actives from formulation.

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GLOSSARY

Angiogenesis: The development of new blood vessels.

Anti-tumorigenic: Tending to suppress tumors.

BTH: Bovine testicular hyaluronidase, EC 3.2.1.35.

DMF: N,N-Dimethylformamide

DMSO: Dimethyl sulfoxide

Endothelial cell: A thin, flattened cell, a layer of them lines the inside surfaces of body cavities, blood vessels, and lymph vessels, making up the endothelium.

ESI-MS: Electrospray Ionization Mass Spectrometry. An analytical technique that can provide both qualitative (structure) and quantitative (molecular mass or concentration) information on analyte molecules after their conversion to ions.

GlcNAc: N-acetyl-D-glucosamine

HA: Hyaluronic acid, hyaluronan.

Inflammation: The end result of the different bodily processes, in response to an injurious agent.

Interleukins: A variety of naturally occurring polypeptides modulating inflammation and immunity.

MALDI-TOF: Matrix-Assisted Laser Desorption/Ionization –Time Of Flight. A method of mass spectrometry in which laser radiation of analytematrix mixture results in the vaporization of the matrix which carries the analyte with it. Ion´s mass to charge ratio is determined via a time measurement.

MALS: Multi-Angle Light Scattering. An analytical technique for determining absolute molar masses, sizes and conformation of all types of macromolecules.

Mitosis: The process where a single cell divides resulting in generally two identical cells, each containing the same number of chromosomes and genetic content as that of the original cell.

Mitogenic: Causing mitosis.

NMF: Natural Moisturizing Factor

Pro-angiogenic: Tending to promote angiogenesis.

Pro-inflammatory: Tending to promote inflammation.

Proliferation: The reproduction or multiplication of cells.

SEC: Size exclusion chromatography. A method in which molecules in solution are separated by their size**.**