INVESTIGATION OF THE EXTRACTION PROCESS OF HYALURONIC ACID FROM NATURAL SOURCES

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Abstract: Properties and methods for obtaining hyaluronic acid and its derivatives from raw material of animal origin are reviewed. The importance and practical application of hyaluronic acid in various fields are discussed.
Keywords: hyaluronic acid, obtaining methods, natural sources.

Introduction

The production and usage of hyaluronic acid (HA) is growing worldwide. In Moldova, hyaluronic acid is less known as pharmaceutical and cosmetic component.

HA is a precious natural biomaterial, biocompatible, safe and non-allergic. For these reasons HA is one of the most "agreeable" cosmetic ingredient. Emulsions based on it have a smooth soft consistency. Products of HA are compatible with human skin and do not cause allergic reactions. Same properties have also sodium, potassium, calcium, zinc, copper and other salts of HA, which are successfully used in cosmetic industry.

In our country we have enough cheap sources that can be used for hyaluronic acid preparation. The problem is to develop economically efficient methods of obtaining and purification of hyaluronic acid and its derivatives in quantities sufficient to be sold and used in the production of other bioactive compounds based on it.

The properties of hyaluronic acid

The name of HA reflects its transparent nature (the Greek word for glass is hyalos) and the content of one of uronic acids (glucuronic acid).

Hyaluronic acid is a compound present in the human body. It is one of the main constituents of the extracellular matrix of connective tissue and it is concentrated in synovial fluids, heart valves, eyes, cartilage.

In pure form HA is a white odorless powder soluble in water and insoluble in organic solvents with high viscosity. Specific rotation in aqueous solutions is –(70–80)°. HA is a polyelectrolyte which $pK_{HA} = 3.21$. A molecule of HA is able to retain about 200–500 molecules of H_2O. It has a high specific hydrodynamic volume. The molecule form depends on the pH and ionic strength of the solution. It was found that as the result of electrophoresis for an ionic force of 0.12 only 14% of ionized groups are effective, and for an ionic force of 0.02 already 80% of these groups are effective [1].

Chemical and spatial structure of the macromolecule, high molecular weight, high viscosity of the solution and hydrophilic qualities are important biological properties of HA. Due to these properties, HA has various biological functions and roles in animal bodies such as: participation in embryogenesis and morphogenesis processes, intercellular relationships and communication, mechanical strength of tissues, reducing friction in biomechanical systems, formation and proper function of cartilages, formation and

maintenance of transparent structures of the eye, the permeability of biological membranes including vascular walls, water retention [2].

Biological researchs have shown no toxic properties, irritating, allergenic. A comparative study was conducted on biocompatibility and safety of HA obtained from three natural sources: umbilical cord, cockscomb and bovine vitreous [3].

The importance and usage areas of hyaluronic acid

Due to its properties, HA is one of the most attractive biomaterials for pharmaceutical and cosmetic industry [4].

Hyaluronic acid provides lubrication and hydration of connective tissues, including those of the skin. In the absence of lubrication and hydration of tissue and lose elasticity if skin leads to wrinkles and creases.

Due to its structure, HA form a film on the skin invisible, transparent and elastic, while acting in depth in the tissue, cartilages and joints. Its role is to keep the most important characteristics of young and healthy skin: suppleness, elasticity and tone. HA capacity to restore the interstitial matrix and liquid skin turgor alterations and wrinkles is successfully used for "rejuvenating" skin.

HA is a glycosaminoglycan, an essential component of extra cellular space, in which the collagen and elastin fibers are suspended. HA has an increased capacity to retain water, like a "sponge" that allows maintaining hydration, elasticity, skin firmness. Unfortunately, body's ability to produce hyaluronic acid decreases with age, and so the skin tissue becomes dehydrated, wrinkled and tonicity diminishes. HA injections are used more than 12 years in Europe and gradually replaced the injection of bovine collagen, which is sometimes complicated and required tests prior allergic reactions. Being a natural filler, it has a very low rate of allergic reactions - 0.06% vs. 3% for collagen.

HA is used to fill wrinkles and lip augmentation. Today the area of its application and usage in aesthetic medicine has expanded greatly, it is frequently used for volumization, nonsurgical facelift, correct dark circles and breast augmentation.

Also, HA is an important component of cartilage. In this role it dampens shocks in the joints, has lubrication effect and protects joints for chronic inflammation (eg arthritis). It is used to successfully heal stretching of ligaments. HA is a common ingredient in anti-osteoarthritis preparations and is frequently injected into joints, being a very effective treatment.

HA helps the immune system, acting as an antioxidant, it increases water retention in tissues, increases lubrication of heart valves, and serves as an adjunct to anti-infection treatment.

In the 1990s, hyaluronic acid began to be used in ophthalmology to treat corneal trauma.

Besides keeping joints lubricated, hyaluronic acid helps water retention in other tissues of the body, providing hydration of collagen and elastin. Interest in the use of HA as bioactive ingredient in skin care products came with the discovery that the volume of him in the skin decreases with age.

Clinically have been proven extraordinary efficiency of hyaluronic acid to fill wrinkles and smoothing. All clinical trials have shown that hyaluronic acid helps heal wounds faster and fading scars.

Currently procedures to inject cross-linked hyaluronic acid to fill wrinkles and lip augmentation, so-called fillers, are used [5]. For this purpose fits and are generally used

products that are marketed as Amalian (Sweden), Perfectha (France) Remake (Italy), Aphrodite Gold (Germany) etc.

Unlike many other biologically active substances, HA shows all its valuable properties at very low concentrations (0.01 to 0.1%), which allows to create effective cosmetics, whose prices will fit producers as well as consumers. This refers to high molecular weight HA, which are now part of moisturizing creams, lipsticks and lip balms, cellulite cream, sunscreen lotions, anti-inflammatory lotion and wound healing [6, 7].

HA content in the human body is an important factor on which the physiological process of aging and immunity of the body depends upon. To strengthen the immune system and prevent various diseases including cancer various "food additives" are prepared in which HA is used as an ingredient or as compound [8, 9]. HA is called "star" of cosmetology, "hope" of rejuvenation and "pledge" of beauty. Benefits of Hyaluronic Acid supplementation – Cosmetic effect: Skin Hydration from the inside out, correcting in this way wrinkles. – Anti-arthritic: lubricate joints, especially knees and hips ones. – Rejuvenate, anti-aging effect: for men and women between 30 and 40 who are beginning to see signs of aging mirror. The effects are felt quickly after first supplementation with hyaluronic acid.

Obtaining hyaluronic acid

Properties of hyaluronic acid are determined by molecular mass, extraction mode, traces of proteins and other proteoglycans that can contaminate preparations obtained [10].

In research conducted hyaluronic acid was obtained from several natural sources of raw material: crest of hens (CH), cockscomb (CC), bovine vitreous body (BVB), bovine umbilical cord (BUC) [11,12]. Obtain preparations of hyaluronic acid was performed according to the scheme of Fig. 1.

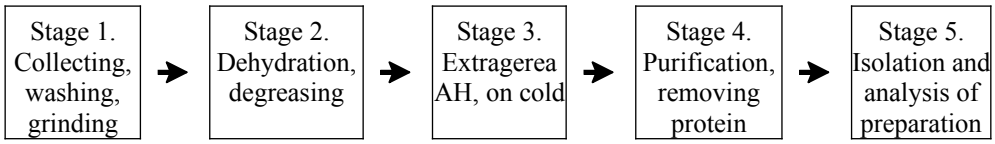

Fig. 1. Process scheme for obtaining hyaluronic acid

In the proposed method the degreasing of raw material is carried out fully before extraction of HA, along with dehydration in Soxhlet apparatus with acetone for 2 hours. HA extraction is performed with NaCl solution only at cold (4–10 °C). From the obtained extract in cold, HA settles with 96% ethanol or acetone. The product is redissolved and proteins are remove by heating-cooling at pH 7 and pH 5 to 5.5 in $CHCl_3$ processing. From the obtained solution is settled and isolated the sodium hyaluronate, HA respectively, with corresponding efficiency.

Characteristics of obtained preparations are vary depending on source of raw materials and obtaining method. For obtained samples was determined the mass part (ω) of hyaluronic acid in the raw material which was subjected to extraction, the protein mass (Lowry method) [13], relative viscosity (Ostwald method) [14] (Tab. 1.).

Table 1. Feature of HA preparations obtained from various sources of raw material

	CH	CC	BVB	BUC
ω (%)	0,5 - 0,7	1,1 - 1,5	0,1 - 0,2	1,4 - 1,8
Proteins (%)	3 - 5	1 - 3	1 - 3	2 - 4
Relative viscosity (η)	11 - 12	12 - 13	5 - 7	12 - 13

Identification of HA in the obtained preparations was made based on the infrared absorption spectra.

Mentioned raw material sources of HA have a different accessibility. Our investigation shows that hen combs and cock combs is preferred.

Taking into consideration the results of research and availability of raw material sources we have agreed to obtain hyaluronic acid from the hen and cock combs collected from local poultry companies [15].

Research result is the issuance process for obtaining HA, increasing the extraction, cost reduction, improving the quality of the final product suitable for use in medical, cosmetic and food.

Obtaining of HA was carried out in several stages as illustrated in Fig. 2.

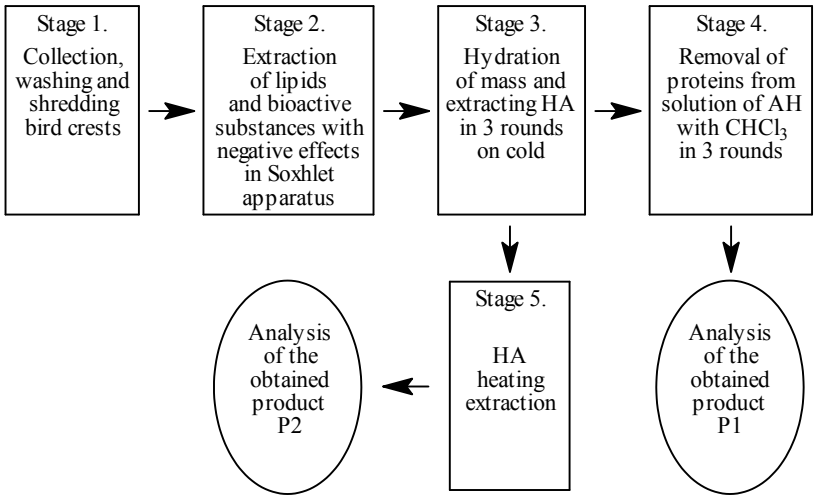

Fig. 2. Scheme process of obtaining and purification of hyaluronic acid, sodium hyaluronate and protein complex of hyaluronic acid

The 1% HA (Product P1) aqueous solution obtained by the proposed method is a viscous liquid, eculent hard, transparent or slightly opalescent, colorless, odorless. Relative viscosity of the solution of 0.1% HA Ostwald Viscometers measured at a temperature of 18 °C was equal to 12.

1% solution absorbance measured at 257 nm HA (absorption maximum nucleotide) and 280 nm (absorption maximum of protein) – is less than 0.1 a (l = 10 mm).

Obtained hyaluronic acid is kept in 96% ethanol, in closed bottles protected from light at 0–4 °C temperature.

The 1% solution of the product P2 (hyaluronic acid-protein complex) presents ultraviolet absorption at a wavelength 266.5 nm equal to 0.609 a. (l = 10 mm).

The resulting products are used in further studies.

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