Reviews on Advanced Materials and Technologies, 2024, vol. 6, no. 2, pp. 67–79
DOI: 10.17586/2687-0568-2024-6-2-67-79

Hyaluronic Acid as Object of Analysis and Accessory Material for X-Ray and Laser Science: a Review

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Article history

Received June 13, 2024
Accepted June 20, 2024
Available online June 30, 2024

Abstract

Modern medicine greatly needs high-effective and safe medications and diagnostic agents. Encapsulation of pharmaceutical agents having low water solubility and lipophilicity into biopolymer matrixes allows to increase the bioavailability of such systems. Hyaluronic acid is one of the most suitable polymer for this purpose. However, in spite of the large amount of drug delivery systems based on it, the structure of such systems is unknown, which hinder the development of high effective therapeutic medication and, as a result, the transition toward personalized medicine. X-ray, laser and synchrotron techniques could help us to understand the interaction between the drug and polymer matrix, that allow to further extend for another biological molecules. This review aims to discuss current status of the previous investigations of materials based on hyaluronic acid via X-ray, laser and synchrotron methods of analysis. Moreover, key information related to hyaluronic acid is provided.

Keywords: Hyaluronic acid; Synchrotron radiation; X-ray; Modern drug delivery systems

1. INTRODUCTION

Nowadays biomedicine and bioengineering desperately need the modern high-effective drug delivery systems based on polymers and their mixture, which could solve the current problems related to the hydrophobic biologically active agents (BAA) and other pharmaceutical substances, such as low solubility, lipophilicity, and, respectively, biological availability and efficacy, being at the same time biosafe and biodegradable with low side-effects and non-toxic degradation products.

Despite the fact, that the polymer systems based on natural biopolymers (e.g., polysaccharides) with the natural BAA is well known, the understanding of the structure and formation mechanism of polymer-BAA complexes (conjugates) has high fundamental significance and practical helpfulness. Moreover, changes during aging degradation and/or usage are very important for an understanding and prediction of drug release rate and kinetic.

One of the possible ways is to use the X-ray, synchrotron and laser techniques for such analysis. These techniques allow understanding of several key issues related to the drug delivery systems, e.g., the conformation of polymer chains involved into the drug delivery systems, the structure of drug loaded into polymer matrix (amorphous or crystalline), the kinetics of conjugate formation between the polymer matrix and BAA, etc. However, these techniques could not be used without other methods, for

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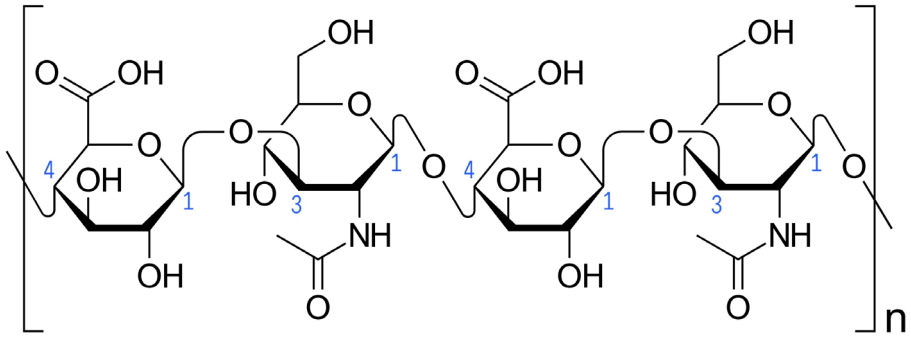

Fig. 1. Hyaluronic acid structure.

instance, physico-chemical (Fourier transform infrared spectrophotometry (FTIR), differential scanning calorimetry (DSC), thermal gravimetric analysis (TGA), etc.), computer modelling techniques, including molecular docking, and other.

This review aims to discuss current status of the recent investigations of materials based on hyaluronic acid using X-ray, laser and synchrotron methods of analysis. Moreover, key information related to hyaluronic acid properties is provided.

2. HYALURONIC ACID AND ITS KEY PROPERTIES

Hyaluronic acid (HA, Fig. 1) is a natural non-sulfated polysaccharide, consisting of the repeating N-acetylglucosamine and D-glucuronic acid [1]. It is known, that more stable form of HA is represented by sodium or potassium salt, where the H-atoms of the carboxylic groups of D-glucuronic acid are substituted with sodium or potassium, respectively [2].

Due to its specific rheological properties, HA solutions could be used as natural biocompatible and biodegradable lubricants, which acts as a natural damping agent. The biological, rheological, physico-chemical, and chemical properties of HA and its derivatives allow to use materials based on it in various biomedical and bioengineering applications, for example, in ophthalmology, otorhinolaryngology, orthopaedy, traumatology [3,4].

HA could be produced from animal source (bovine vitreous body, rooster's combs) and by bacterial fermentation [5].

HA and its salts (except for Ca- and Mg-) have good solubility in water and aqueous solutions, however, insoluble in most organic solvents, which hinders processing of the biopolymer and its solutions [6].

In solution, the HA chains exist in the form of an extended coiled helix. Moreover, HA chains become intertwine with each other at very low concentrations. At higher concentrations, the solutions have a high level of dynamic viscosity, but shear-depending (non-Newtonian character). The viscosity of the polymer solutions drastically depends on the polymer concentration and its molecular weight [7,8].

The primary structure of HA consisting of two disaccharide units has five H-bonds existing between each two adjacent disaccharides (Figs. 2 and 3). Water molecules could act as a “bridge” between H-bonds belonging to the same or neighboring HA chains. Tertiary structures are formed as double-layered ribbon-shaped spirals by twisting each disaccharide unit by 180° compared to those at the front and back of the chain [9].

The presence of a large number of OH-groups explains the ability of HA to hold a lot of water (up to 1000

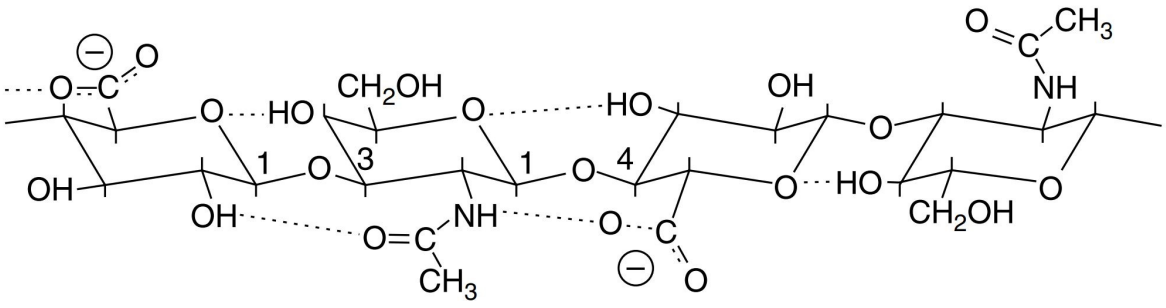

Fig. 2. The schematic illustration of H-bonds formed in HA. Reproduced with permission from Ref. [6], © 2015 John Wiley and Sons.

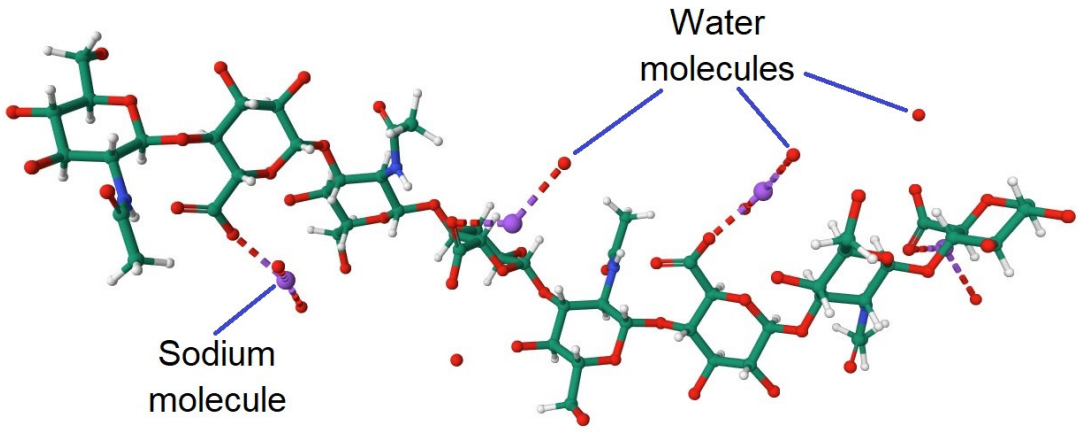

Fig. 3. The hyaluronic acid sodium salt structure in water solution. Adapted from Protein Data Bank (www.rcsb.org).

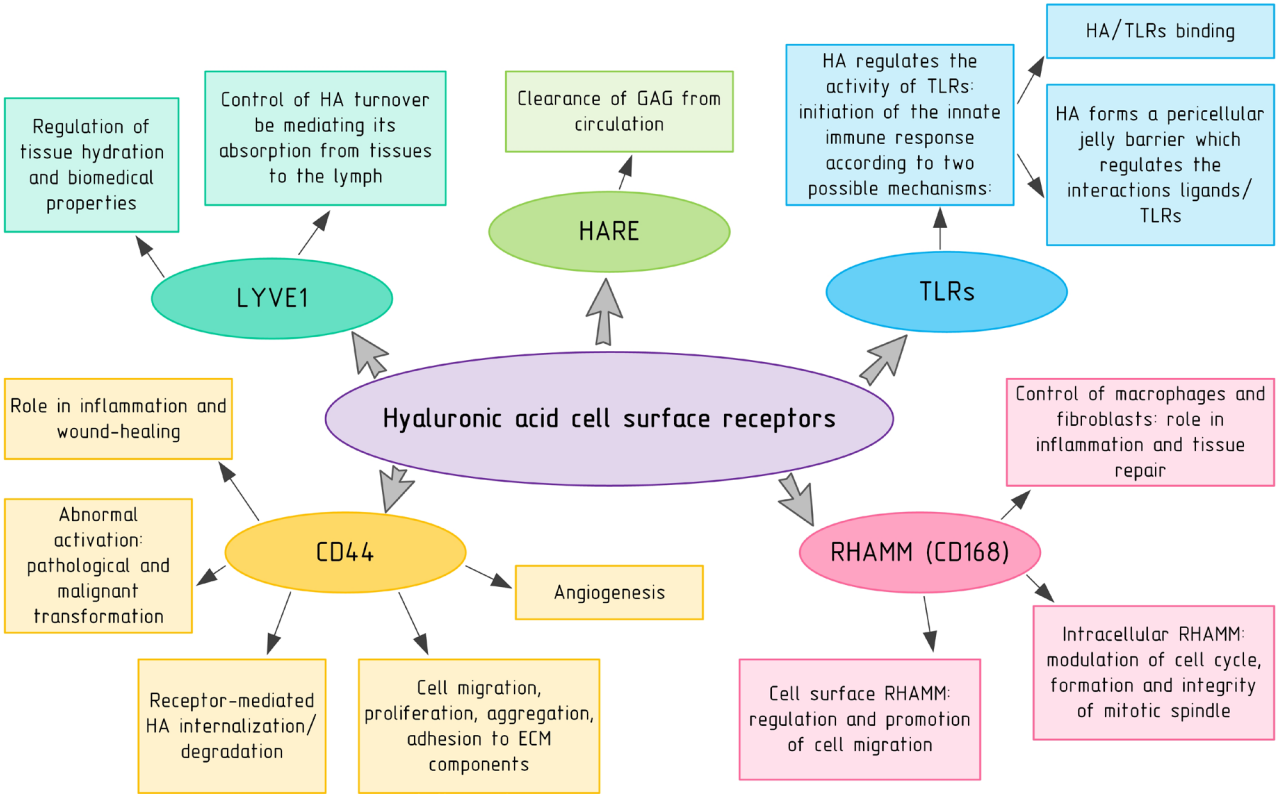

Fig. 4. Hyaluronic acid cell surface receptors.

times w/w). Water holding and polymer swelling predetermines the biological functions of HA in the regulation of tissue permeability and its barrier properties [10].

Moreover, HA binds to various cell receptors, thereby triggering different biological responses (Fig. 4) [11]. The loading of bioactive compounds and pharmaceutical agents into the polymeric matrix makes it possible to regulate the effects and provide targeted therapies [12].

The HA-binding domain of murine CD44 (Type A and Type B) complexes with an 8-mer HA are demonstrated in Figs. 5 and 6, respectively.

As mentioned above, HA takes part in various key biochemical processes in the living organisms. The ability to hold water molecules allows HA to act as a "living" barrier when the fluid content of tissues changes. HA acts as a filler of cellular and intercellular space and, given its rheological properties, it, along with the specific glycoprotein lubricin [13], also performs the function of lubricating joints [14]. HA not only performs structure-forming and barrier functions, but also plays a key role in the inflammation process and immune response, as well as tissue regeneration [15]. Due to its unique biological and physicochemical properties, HA is being actively studied as a promising biomaterial for various medical, pharmaceutical, food and cosmetic applications. One of the topical application areas of HA is a biocompatible polymer carrier

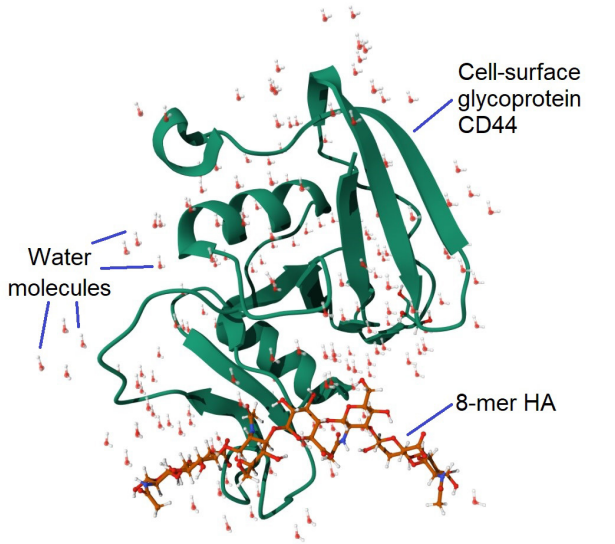

Fig. 5. The HA-binding domain of murine CD44 (Type A) complexes with an 8-mer hyaluronic acid. Adapted from Protein Data Bank (www.rcsb.org).

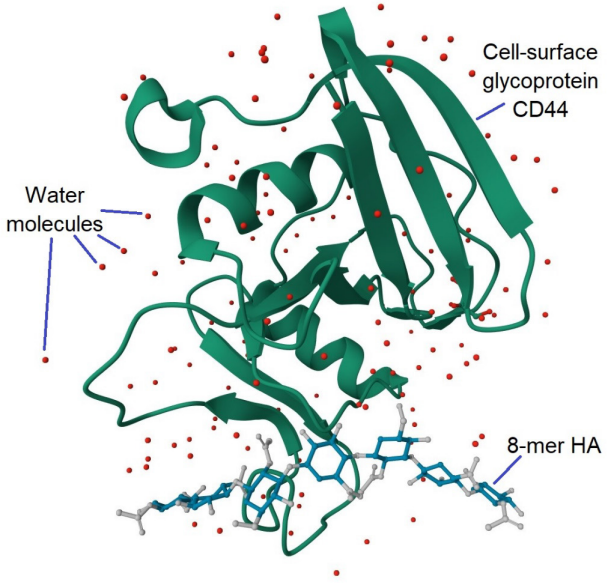

Fig. 6. The HA-binding domain of murine CD44 (Type B) complexes with an 8-mer hyaluronic acid. Adapted from Protein Data Bank (www.rcsb.org).

in drug and vaccine targeted delivery systems [16], as well as in diagnostic systems [17]. In addition to drugs and biologically active substances, transplantable cells can be encapsulated in a shell of HA [18], which increases the relevance of research in this area, opening up the possibility of transition to personalized medicine.

The use of HA as a drug carrier for the treatment of tumors is based on the fact that, by binding to specific receptors, it participates in the activation of intracellular signaling cascades associated with tumor growth and tumor cell adhesion [19]. It was previously found that overexpression of the CD44 receptor correlates with increased metastasis formation [20]. The mechanism of action of drug-loaded HA was proposed by Gibbs et al. [21]. The effectiveness of HA as a drug carrier for irinotecan for the treatment of metastatic colorectal cancer was confirmed in a randomized clinical trial (phase II) [21]. The drug system used in clinical trials was fabricated by mixing of 1.0 wt.% aqueous solution of HA (MW 825 kDa) and irinotecan with subsequent dilution in 5 wt.% glucose solution (total volume was equal to 500 ml). The obtained solution was infused through an intravenously route of the administration over 90 min. However, the structure of possible HA-irinotecan complex was not analysed.

There are many varieties of drug delivery carriers, such as liposomes, dendrimers, hydrogels, magnetic nanoparticles, quantum dots, polymer nanoparticles, etc. [22]. Polymer nanoparticles are particles derived from natural or synthetic polymers ranging in size from 1 to 1000 nm [23]. The nano-sized level of particles contributes to their better absorption by cells (in comparison with microparticles from 1.0 to 10.0 μm) and provide a prolonged mechanism of action [24]. Biodegradability, non-toxicity, and biocompatibility of natural polymers determine the effectiveness and safety of such nanoparticles as a carrier for drug delivery, which, compared to non-encapsulated drugs, has reduced systemic exposure and fewer side effects.

It is known that particles containing HA have a negative electrokinetic or zeta potential (ζ-potential) [25], the value of which can be –30 mV or lower. The negative ζ-potential ensures particle stability and prevents the formation of aggregates, which allows such particles to be used for direct injection without the risk of blocking blood vessels and capillaries.

The use of natural biologically active substances as pharmaceutical agents is more promising due to their wide range of therapeutic activities, high efficiency and low side effects. However, most of these biologically active substances have a hydrophobic nature and low bioavailability, which make their use difficult. Using the biopolymers, in particular, HA, as a drug carrier allows increasing water solubility and improving bioavailability of encapsulated substance.

3. X-RAY AND LASER TECHNIQUES

3.1. Dynamic light scattering

Dynamic light scattering (DLS) technique could be used not only for nanoparticles diameter and distribution analysis (hydrodynamic size, polydispersity index PDI), but also for stability characterization of obtained structures. Moreover, the equipment for DLS is usually

Table 1. Morphological and electrical characterization of the nanoparticles. Reproduced from Ref. [26], © 2019 Gennari et al., published by Beilstein-Institut. Available under the terms of CC BY 4.0 license.

Sample name	Z-average size (nm)	PDI	ζ-potential (mV)
$Chit_{35}$-TPP	166 ± 5	0.17 ± 0.05	+37
$Chit_{35}/HA_{templ}$	310 ± 50	0.17 ± 0.06	−38
$Chit_{35}/HA_{dir}$	220 ± 30	0.19 ± 0.07	−39
$Chit_{650}$-TPP	368 ± 15	0.28 ± 0.01	+50
$Chit_{650}/HA_{templ}$	320 ± 30	0.17 ± 0.06	−38
$Chit_{650}/HA_{dir}$	260 ± 40	0.20 ± 0.05	−40

Chit – chitosan
HA – hyaluronic acid
TPP – sodium tripolyphosphate
templ – template-based techniques of nanoparticles fabrication
direct – direct complexation method of nanoparticles fabrication

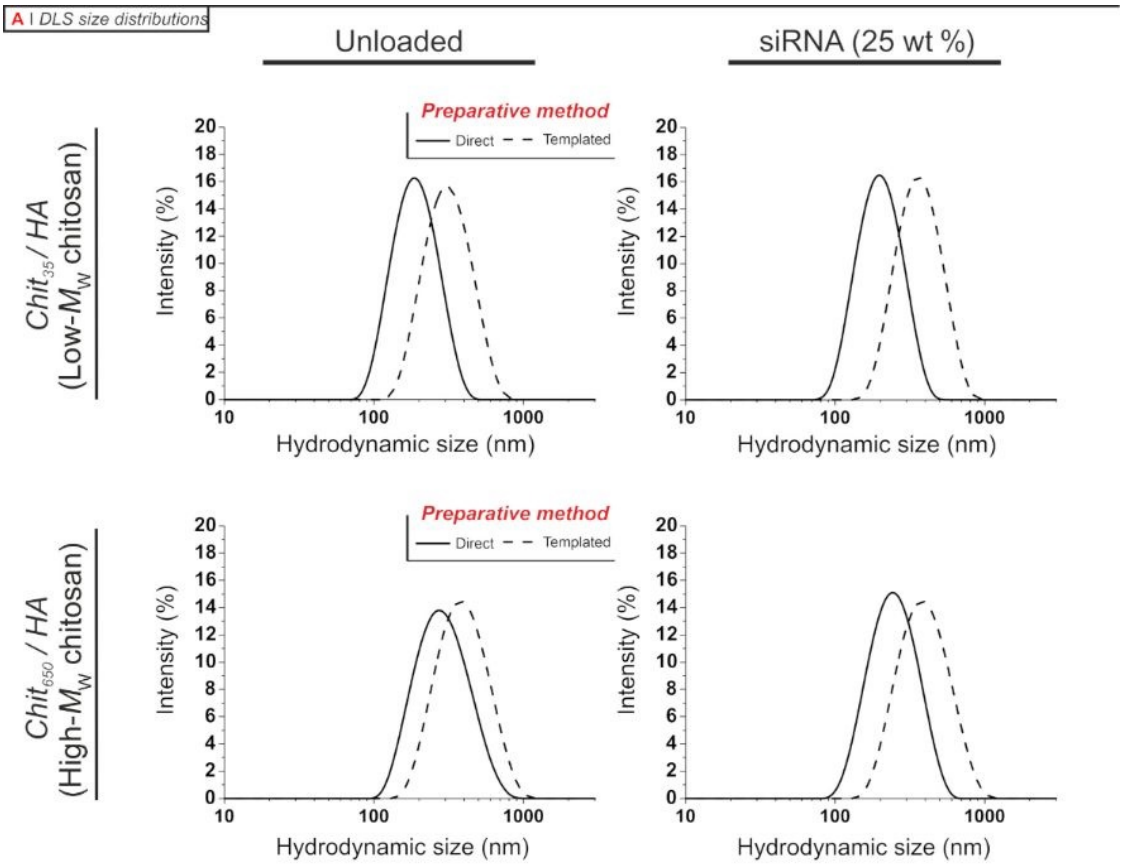

Fig. 7. Size distribution of HA/chitosan nanoparticles prepared from chitosan with different molecular weight (35 and 650 kDa) by template-based method and direct complexation with or without siRNA. Reproduced from Ref. [26], © 2019 Gennari et al., published by Beilstein-Institut. Available under the terms of CC BY 4.0 license.

attributed to the ζ-potential measuring instrument, which is very important for characterization of modern drug delivery systems.

For example, Gennari et al. [26] obtained nanoparticles based on HA and chitosan by two methods: template-based method and direct complexation. In this case the authors used the ZEN3600 Zetasizer Nano ZS instrument equipped with a solid state HeNe laser with λ = 633 nm at a scattering angle of 173°. Results are demonstrated in Table 1 and Fig. 7. The authors showed that there were no significant differences between the nanoparticles obtained by two various methods. At the same time, there were differences in internal compactness and aggregation: in case of template-based method, the nanoparticles had tendency to aggregation and less compact structure than nanoparticles were obtained by direct complexation technique.

Table 2. Z-average diameters, PDI, and zeta potential ζ of CS-TPP/functionalized hyaluronan nanoparticles in relation with HA degree of substitution (DSHA). Reproduced from Ref. [27], © 2022 The Authors, published by MDPI, Basel, Switzerland. Available under the terms of CC BY 4.0 license.

Synthon	DSHA [%]	Z-Average ± sd (nm)	PDI ± sd	ζ ± sd (mV)
PEG339	2.4	132 ± 2	0.19 ± 0.01	+26 ± 3
	7.6	128 ± 1	0.18 ± 0.02	+23 ± 4
	15.7	138 ± 2	0.19 ± 0.01	+24 ± 3
	38.9	128 ± 1	0.17 ± 0.01	+29 ± 3
PEG2000	1.3	141 ± 2	0.18 ± 0.02	+26 ± 4
	7	149 ± 1	0.18 ± 0.01	+21 ± 3
	14.1	137 ± 2	0.19 ± 0.02	+24 ± 4
	32	146 ± 1	0.18 ± 0.01	+24 ± 3
TFB	5.2	153 ± 3	0.18 ± 0.02	+28 ± 3
	6.5	140 ± 2	0.20 ± 0.01	+25 ± 4
	15.6	148 ± 3	0.18 ± 0.02	+23 ± 3
	29.2	146 ± 3	0.20 ± 0.01	+23 ± 3
Rhod	0.3	137 ± 3	0.18 ± 0.01	+22 ± 3
	1.2	147 ± 2	0.18 ± 0.02	+23 ± 4
	2.8	148 ± 3	0.20 ± 0.01	+23 ± 3
	4.9	141 ± 3	0.18 ± 0.02	+21 ± 4
No synthon	0	139 ± 2	0.18 ± 0.01	+22 ± 3

Table 3. DLS measurements at various storage time. Reproduced from Ref. [28], © 2022 The Authors, published by MDPI, Basel, Switzerland. Available under the terms of CC BY 4.0 license.

Formulation	Mean Diameter (nm)	PDI	ζ (mV)	Time
PP-NPs	109 ± 11	0.11 ± 0.04	−26 ± 6	T0
HA-PP-NPs	145 ± 16	0.14 ± 0.076	−34 ± 8	
PP-NPs	118 ± 15	0.13 ± 0.05	−26 ± 5	T7
HA-PP-NPs	135 ± 23	0.15 ± 0.05	−24 ± 4	
PP-NPs	117 ± 10	0.16 ± 0.05	−23 ± 2	T14
HA-PP-NPs	142 ± 17	0.13 ± 0.06	−29 ± 4	

Another study [27] used protonated form of hyaluronic acid (HAp) and obtain functionalized derivatives of HA with following radicals: n-octylamine C_8-NH_2, 1,1,1-trifluoropropylamine TFP-NH_2, 2,5,8,11,14,17,20-heptaoxadocosan-22-amine PEG_{339}-NH_2, methoxy-poly(ethylene)glycol-amine PEG_{2000}-NH_2, and amine-functionalized rhodamine Rhod-NH. Moreover, the authors fabricated nanoparticles based on these derivatives and studied its properties. The results of morphological analysis are demonstrated in Table 2.

La Verde et al. [28] synthesized HA-coated poly(lactide-co-glycolide) (PLGA) nanoparticles and characterized them in details, especially after shelf aging at 4 °C during 7 days and 14 days of storage. The authors also used the Surface-Enhanced Raman Spectroscopy (SERS) method for external shell characterization, which is very useful for the analysis of nanoparticles for biomedical, bioengineering, and biotechnological applications.

The results of DLS measurements of obtained nanoparticles at various storage times are shown in Table 3.

SERS allows analyzing the physico-chemical properties of nanoparticles [28], however, it does not display the data on the chemical nature related to these properties. This method is recommended for the characterization of multilayer nanoparticles and their external layer (shell). The comparison of Raman and SERS analysis of nanoparticles is presented in Fig. 8.

As shown in Fig. 8, the Raman spectrum has significant differences from the SERS spectrum. Thus, SERS could be used for the analysis of coated nanoparticles. At the same time, the authors highlighted, that this method allows to use very low power and short exposition, preventing the photo- and thermal

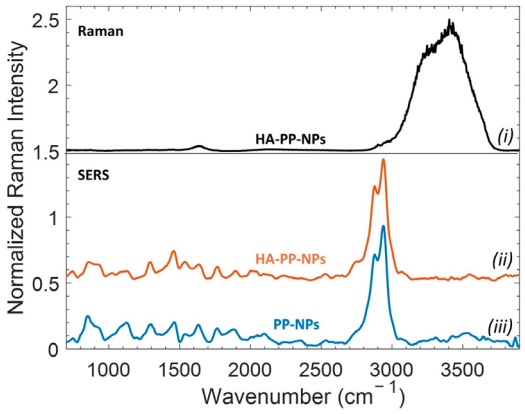

Fig. 8. Raman and SERS spectra of HA-PP-NP (i, ii) and PP-NP (iii). Reproduced from Ref. [27], © 2022 The Authors, published by MDPI, Basel, Switzerland. Available under the terms of CC BY 4.0 license.

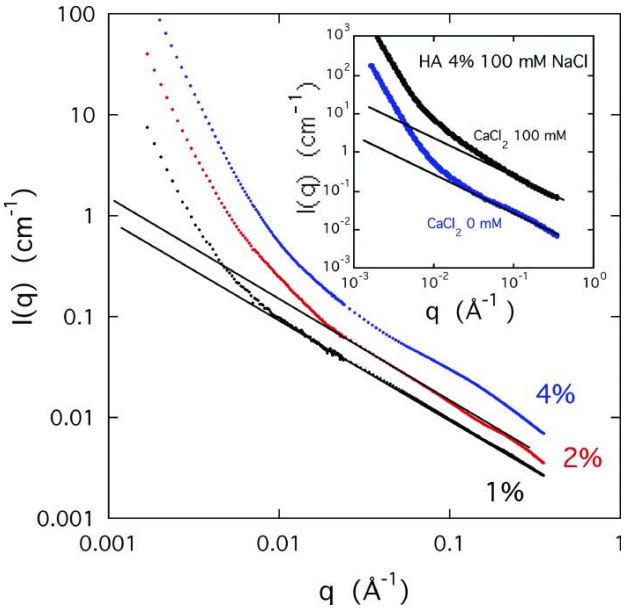

Fig. 9. SAXS curves for hyaluronic acid solutions at three concentrations in 100 mM NaCl. Inset: SAXS response of 4% HA solutions in 100 mM NaCl (lower curve) and in 100 mM NaCl + 100 mM $CaCl_2$ (upper curve). Reproduced with permission from Ref. [32], © 2009 AIP Publishing.

degradation of the samples, which is very important for natural-based components.

3.2. Analysis of the HA conformation into solutions

As noted above, HA has a unique primary, secondary, and tertiary structure, and the understanding of this structure and its changes under the influence of various conditions is very important for the investigation of modern biomedical and bioengineering applications.

Thus, small angle X-ray scattering measurements (SAXS) allows to determine the effect of various ions in polymer solution on the HA molecular conformation or organization of the polymer chains [29]. At the same time, anomalous small angle X-ray scattering (ASAXS) could help to analyze the ion distribution near the polymer [30]. Interestingly, the ASAXS method demonstrated that counter-ion forming a dense shell layer of spatial extent of the counter-ion cloud is much smaller than for monovalent counter-ion around the polymer [31].

Horkay et al. [32] analyzed various aqueous HA solutions with polymer concentration from 0.5% to 4% (by weight). Moreover, solutions contain NaCl (50 or 100 mM) and $CaCl_2$ (from 0 to 200 mM) to analysis of the HA conformation alterations under the salt addition.

Fig. 9 shows the SAXS response for HA solutions with polymer concentration equal to 1.0 wt.%, 2.0 wt.%, and 4.0 wt.% in 100 mM NaCl. The inset curves demonstrate that the "dopant" of 100 mM $CaCl_2$ at polymer solution with HA concentration equal to 4.0 wt.% produces insignificant alteration in the scattergram. According to the obtained results, the SAXS could be the evidence that HA polymer chains in the semi-diluted solutions have an elongated scapiform (rod-shaped) structure. Note that this structure is very stable and did not change even at high ionic force exposed with uni- and divalent ions into solution.

Exogenous hydrogels allow to regulate the physico-mechanical properties of cells and cell walls, as well as tissues, and can regulate and even inhibit various biological and pathophysiological processes. Smart hydrogels based on biopolymers can alternate their properties under the influence of environmental exposure (pH, temperature, ions, etc.). The analysis of the macroscopic response of such hydrogels and molecular interactions is a challenging task that allows predicting and tuning the properties of hydrogels.

Two-dimensional infrared spectroscopy (2D-IR) is one of the several suitable techniques for analysis of the interactions at the macromolecular scale. 2D-IR allows resolving molecular couplings and dynamics at a short time scale. It is known that hydrogen bonds or electrostatic forces usually change on a time scale equal to picoseconds (10^{-12} s), and with 2D-IR, "snapshots" of these interactions can be "frozen" with femtosecond (10^{-15} s) time resolution.

Giubertoni et al. [33] use 2D-IR to study the pH-induced gelation of HA which has a transition from a viscous to an elastic state in a short pH range equal to 2.5 ("putty state", Fig. 10).

2D-IR spectroscopy measurements (Fig. 11) show that the significant pH-associated gelation of HA at pH 2.5 involves the enhanced formation of double hydrogen bonds between carboxylic (–COOH) and amide (–$NHCOCH_3$) groups, and of strong single hydrogen bonds between a carboxylate anion (–COO^-) and amide groups. The enhanced formation of H-bonds is intimately related and even enabled by changes in the structure and electrostatic

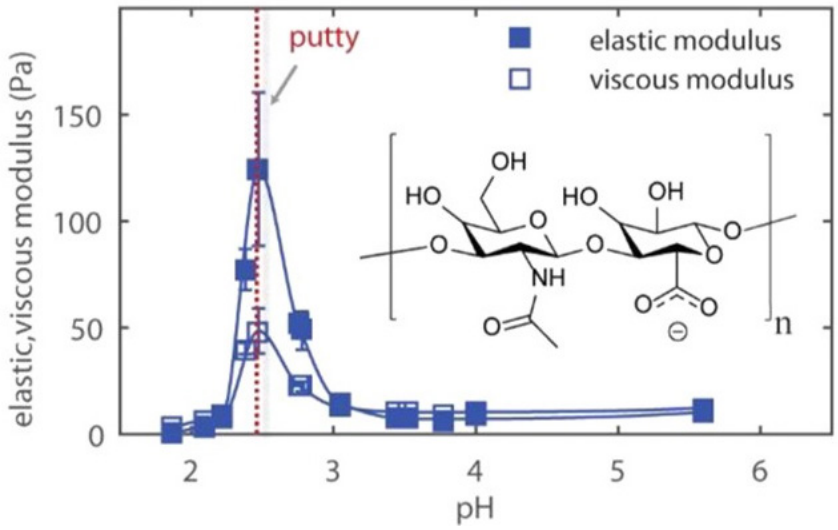

Fig. 10. The pH dependence of the viscous and elastic modulus of hyaluronic acid in D_2O solutions. A sharp peak in the elastic shear modulus at pH 2.5 is observed. Reproduced from Ref. [33], © 2019 American Chemical Society. Available under the terms of CC BY-NC-ND 4.0 license.

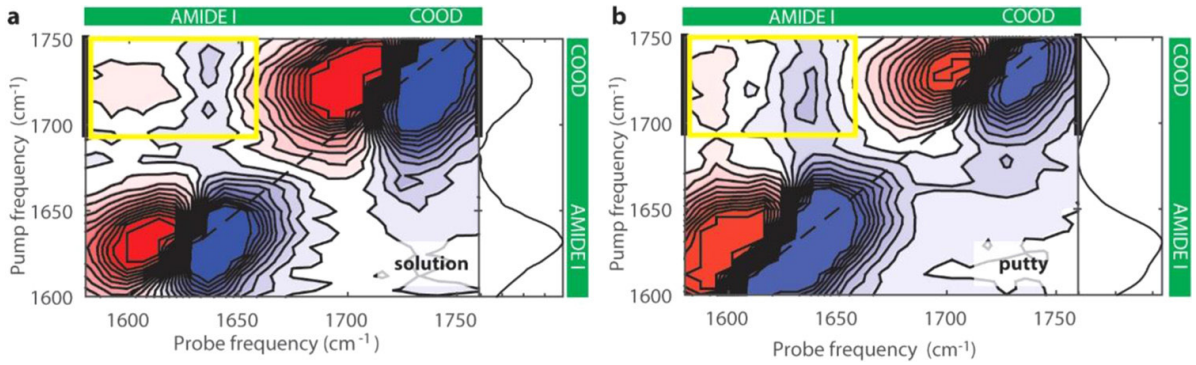

Fig. 11. Isotropic two-dimensional infrared spectra of solutions of hyaluronic acid in D_2O at a concentration of 20 mg/mL in the spectral region of the carboxylic acid (ν_{COOD}) and the amide I ($\nu_{AM.I}$) vibrations. 2D-IR spectra (a) for the solution state (pH = 1.6) and (b) for the "putty state" (pH = 2.5). Delay time Tw of 1.5 ps. The cross-peaks are indicated by the yellow rectangles and show the presence of vibrational interactions between $\nu_{AM.I}$ and ν_{COOD}. The thick black lines represent the integrated pump region used for the fitting. The pump and probe spectra are centered at 1680 cm^{-1}. Reproduced from Ref. [33], © 2019 American Chemical Society. Available under the terms of CC BY-NC-ND 4.0 license.

interactions of the HA chains. These strong interactions can only exist if the carboxylic acid groups are partially deprotonated, which explains the narrow pH range of putty state.

3.3. Characterization of the interactions between HA and BAA

It is known that HA is a promising polymer for the modern drug delivery systems [34]. It is obtained by encapsulation of the hydrophobic BAA resulting in increasing the solubility and bioavailability of the latter [35]. Despite the great number of polymer systems elaborated and produced, the formation mechanism of such complexes is not always could be evaluated by traditional physico-chemical methods of analysis like FTIR, Raman, UV-vis spectroscopy etc. The X-ray scattering allows making a deep analysis of polymer-BAA interactions and helping the scientists to understand the structure of such topical complexes [36].

Camptothecin being a topoisomerase inhibitor could be highlighted as a promising anticancer agent, which inhibits replication and transcription, kills cells in the S-phase of the cell cycle, blocks the cell cycle at the G2 stage, and causes fragmentation of chromosomal DNA [37]. Unfortunately, camptothecin is insoluble in water and has low efficacy and availability. For the increasing of its solubility the conjugates with polymer could be made, resulting in, for example, polymer nanoparticles based on HA [38]. Note, that camptothecin was used together with doxorubicin in special ratio in accordance with "Doxorubicin and Camptothecin Tailored at Optimal Ratios" called in literature "DOCTOR".

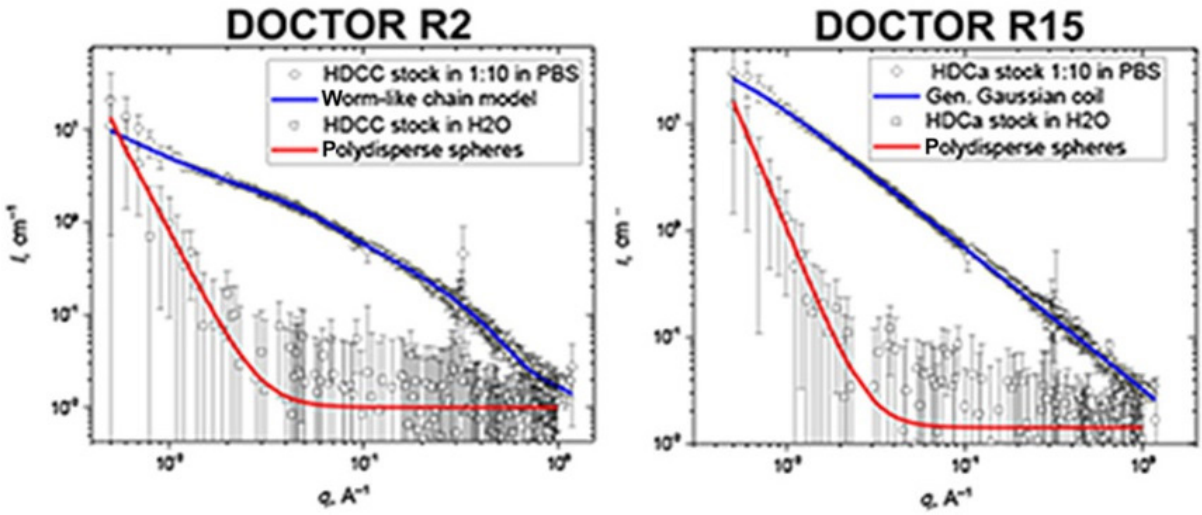

Fig. 12. SAXS curves for "DOCTOR" conjugates in distilled water and phosphate buffered saline. Here R2 and R15 are the molar ratios of doxorubicin: camptothecin. Reproduced from Ref. [38], © 2021 The Authors, published by American Association for the Advancement of Science. Available under the terms of CC BY-NC 4.0 license.

SAXS analysis was made for the determination of the structure of "DOCTOR" in distilled water and phosphate buffered saline [38]. SAXS demonstrates minimal or absent scatter for polymer samples in distilled water (Fig. 12, red curve). At the same time, the scattering intensity was higher by a hundredfold of magnitude in phosphate buffered saline, which could prove the spherical shape of "DOCTOR" (Fig. 12, blue curve). Author noted that a similar tendency was detected in case of single drug-loaded polymer systems.

It was found that "DOCTOR" exists as monomers or molecularly dispersed short constituents that fit alongside with a small fraction of the aggregates with the dimensions equal to 100 Å in water. SAXS highlights the possibility of existing self-assembling polymeric nanoparticles based on HA having the three types of interactions: electrostatic repulsion, hydrophilicity, and hydrophobicity [38].

Apart from the hydrophobic agents, the hydrophilic drugs also could be loaded into the HA matrix. Thus, Camara et al. [39] obtained the dexamethasone-loaded nanoparticles based on HA.

Fig. 13 demonstrates SAXS and wide-angle X-ray scattering (WAXS) profiles of the hyaluronan-dexamethasone nanoparticles. SAXS analysis highlights that, on the mesoscale, the formation of the polymer nanoparticles could be exactly characterized by a dexamethasone-filled center (core) which is stabilized by the HA polymer chains. Interestingly, that biopolymer chains fractionally included in the nanoparticle core and partially surrounded the nanoparticle. A core diameter is equal to 200 nm, shell thickness is equal to 1.5 nm.

WAXS allows analyzing the experimental crystal structure of dexamethasone loaded into the polymer nanoparticles. Thus, dexamethasone has crystalline structure with lattice parameters equal to: $a = 10.36$ Å, $b = 16.16$ Å, $c = 23.20$ Å, which corresponds with the previous data [40].

3.4. Analysis of interactions of HA-complexes with the cell receptors

To analyze the ability of the polymers to bind various cell receptors the surface plasmon resonance (SPR) could be used [41]. As mentioned above, there are several key receptors of HA: CD44, HARE, LYVE-1, TLRs, and RHAMM (CD168) [42].

At the same time X-ray scattering measurements could be applied also to determine the structure of complexes consisting of pharmaceutical drugs (or antibodies), HA, and its cell receptors. For example, Škerlová et al. [43] studied the hyaluronate-binding domain (HABD) which is included into the extracellular domain of CD44 and murine monoclonal antibody MEM-85.

The obtained results show that MEM-85 could not directly contend with the binding of HA, but could function through an allosteric and relay-like mechanism. Thus, it binds to the C-terminal extension lobe region of CD44 HABD and stimulates a conformational transposition, which is further overspread into the HA-binding cavity. Antibody MEM-85 initiates a structural transition of CD44 receptor from "well-ordered" into "demi-disordered" form, by analogy the one appearing upon HA binding, resulting in CD44 sloughing from the cell surface.

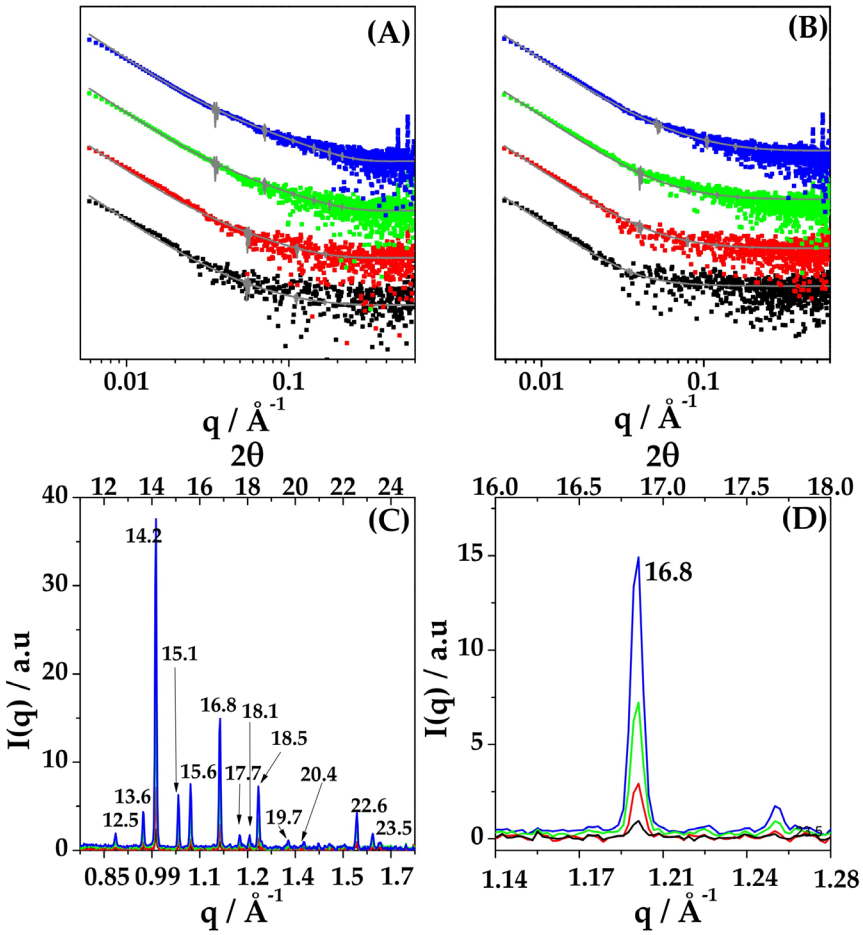

Fig. 13. (A,B) SAXS intensity profiles of the hyaluronan-dexamethasone nanoparticles after subtraction of the intensity contribution of unbound hyaluronan in water (A) and in phosphate buffered saline (B). Lines are the fit with a core-shell polydisperse spherical model. (C,D) WAXS spectra of hyaluronan-dexamethasone nanoparticles in water in the full q range (C) and in the region of one diffraction peak (D) to better visualize the increase of the intensity with concentration. Labels report the position of peaks expressed in 2θ. Hyaluronan-dexamethasone concentration: 1.4 mg/mL (black), 2.8 mg/mL (red), 5.6 mg/mL (green) and 11.2 mg/mL (blue). Reproduced from Ref. [39], © 2021 The Authors, published by MDPI, Basel, Switzerland. Available under the terms of CC BY 4.0 license.

4. HYALURONIC ACID AS AN ACCESSORY MATERIAL

HA could be used not only as a key component for the bioinspired systems, but also as a suitable accessory material for ultrashort X-ray science, for example, for serial femtosecond crystallography of oil-sensitive proteins.

Thus, Sugahara et al. [44] demonstrated the possibility of the use of the water-based HA matrix instead of grease matrix for the analysis of proteinase K and lysozyme protein crystals, which had dimensions equal to 5–10 μm and 7–10 μm, respectively.

It is known that viscous media should be used for the liquid jet injection of small protein crystals. Microextrusion of specimens using viscous media such as the lipid cubic phase, grease, petroleum jelly (Vaseline) allows to maintain a stable stream at a relatively low flow rate (0.02–0.5 μL/min), which helps to decrease sample consumption. However, the presence of the viscous media requires the usage of stronger X-ray scatterings, which can increase the level of background noise. At the same time, viscous media can cause cracking and dissolution of protein crystals caused by physical or chemical processes.

The authors compared several viscous media: mineral oil-based AZ grease, synthetic grease Super Lube and HA water solution (Fig. 14).

Using the matrix based on HA as a general carrier of protein microcrystals for serial sample loading in serial femtosecond crystallography results in the successful analyzing of the structures of two proteins (proteinase K and lysozyme) at resolution equal 2.3 Å in 5–10 μm microcrystals. Note, that the quantity of the samples was less than 1 mg, and analysis can be carried out at the room temperature.

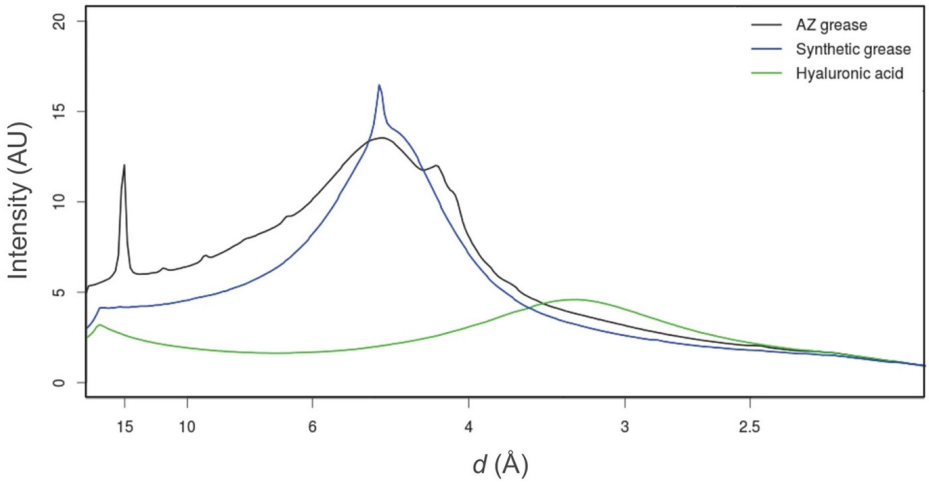

Fig. 14. The average background scattering intensities of ~2,000 images from each matrix. AZ grease, Super Lube synthetic grease and hyaluronic acid are depicted by the black, blue and green lines, respectively. Reproduced from Ref. [44], © 2016 The Authors, published by Springer Nature. Available under the terms of CC BY 4.0 license.

Oil- and aqueous-based crystal carriers are necessary and can be applied for a serial femtosecond crystallography of different proteins. The sample loading methodology with a viscous medium could help to decrease the sample consumption and background noise, which is very important for serial millisecond crystallography using synchrotron, X-ray radiation and free-electron lasers. Serial femtosecond crystallography gives new options for time-resolved studies of light-driven structural changes and chemical dynamics.

5. CONCLUSION

Nowadays, modern drug delivery systems are necessary for the highly effective therapy of various diseases, including cancer. In spite of the fact that there are plenty of elaborated systems based on biopolymers and biologically active agents, the information related to the structure of such systems is limited, which hinders further investigation in his field. In this review article we demonstrate that such methods as DLS, Raman spectroscopy, X-ray scattering and their modifications represent the suitable tools for the structure investigations for the understanding key mechanism of drug-polymers complexes formation. This knowledge is necessary for the prediction of release profiles in the body as well as for further study directed on the introduction of biologically active molecules into drug delivery system to provide targeted mode of action.

ACKNOWLEDGEMENTS

This work was carried out with the support of the Ministry of Science and Higher Education of the Russian Federation (agreement No 075-15-2021-1349).

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УДК 547.995.15:543.42:539.26

Гиалуроновая кислота как объект исследования и вспомогательный материал для рентгеновской и лазерной науки: обзор

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Аннотация. Современная медицина остро нуждается в высокоэффективных и безопасных лекарственных и диагностических средствах. Инкапсулирование фармацевтических агентов, обладающих низкой растворимостью и липофильностью, в биополимерные матрицы позволяет повысить биодоступность таких систем. Гиалуроновая кислота является одним из наиболее подходящих полимеров для этой цели. Однако, несмотря на большое количество систем доставки лекарств на её основе, структура таких систем недостаточно изучена, что ограничивает разработку высокоэффективных терапевтических препаратов и, в конечном итоге, сдерживает переход к персонализированной медицине. Рентгеновские, лазерные и синхротронные методы могут помочь исследователям понять взаимодействие между лекарством и полимерной матрицей, что позволит в дальнейшем исследовать другие биологически активные молекулы. Целью данного обзора является обсуждение современного состояния исследований материалов на основе гиалуроновой кислоты рентгеновскими, лазерными и синхротронными методами анализа. Кроме того, предоставляется ключевая информация, касающаяся гиалуроновой кислоты.

Ключевые слова: гиалуроновая кислота; конформация; рентгеновское излучение; синхротронное излучение; современные системы доставки лекарств