Study of hyaluronan films swelling by water solutions of Hofmeister series ions

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2. **H. Muta et al., Polymer 42 (2001) 6313-6316.**
3. **J. L. Duda et al., J. Phys. Chem. 73(1) (1969) 141-149.**

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ABSTRAKT

Kyselina hyaluronová je polymer složený z disacharidových jednotek N-cetyl-D-glukosamidu a kyseliny D-glukoronové, který reguluje hydrataci a pohyb makrobuněk v tkáních. Jako efektivní léčebný prostředek se uplatňuje v celé řadě medicínských oborů např. oftalmologii, ortopedii či revmatologii. Při použití čisté HA dochází k její degradaci, které se snažíme zabránit přídavkem iontů, konzervačních látek. Tyto konzervační látky se určitým způsobem podílejí na strukturních změnách kyseliny hyaluronové, které jsou nežádoucí a můžeme je potlačit přídavkem iontu z Hoffmeistrovy řady. Cílem této práce byla snaha blíže specifikovat, jak ionty z Hoffmeistrovy řady ovlivňují hyaluronan, a to především při bobtnání.

Klíčová slova: hyaluronan, bobtnání, Hoffmeistrova řada,

ABSTRACT

Hyaluronic acid, also called hyaluronan is a linear polymer composed of a repeating disaccharide containing D-glucuronic acid and N-acetyl-D-glucosamine, which regulated hydration and movement of cells inside of tissues. HA is used as a medicament in a different medicine section for example ophthalmology or orthopaedic medicine. At using the pure form of HA in solution causes a sequent degradation of her structure. This degradation can be decreased by conserving substances and their addition happened to structure changes of HA in solution. These changes can be reduced by Hofmeister ions support hydrophilic interactions. Aim of bachelor thesis will be study of specifications the influence of ions from Hoffmeister serie.

Keywords: hyaluronan, swelling, Hoffmester serie,

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I declare, that the bachelor thesis was written by my person and all used cited references were cited. I agree that with the results of this work can be handled according to judgement of the tutor of bachelor thesis.

In Zlín,

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CONTENTS

INTRODUCTION

The sodium salt of hyaluronic acid called as hyaluronan (HA) is high molecular biopolymer which is part of group polysaccharide occurs in human and animals tissues. In human body HA can be found in cells membranes or body fluids. In structure HA have hydrophilic and hydrophobic properties influent interaction in solutions. Water solutions of HA are very viscous. The most important property of hyaluronan is viscoelasticity. HA is a very important part of the physiology of skin, because it supports her function. Hyaluronic acid is used in medicine and pharmacological industry. The major application is in ophthalmology, wound healing, orthopaedic medicine and recently in cancer therapeutics. The main aim this thesis was definite what influence has ions of Hofmeister series have on the swelling process by hyaluronan. These ions have a great influence on structure and stability of HA in water solution.

I. THEORETICAL PART

1 GENERAL CHARACTERISTIC OF HA

Hyaluronic acid, also called hyaluronan (HA) is part of group anionic polysaccharides that incident in various human and animal tissues and fluids. Hyaluronan is a high molecular linear polymer. The molecular conformation of this biopolymer is strongly dependent on the nature of the counter ions. [1] In the human body, hyaluronan can be found in cells, body fluids, mucous membranes, joints, and spinal disks. Hyaluronic acid (podium salt hyaluronate)-HA is a polysaccharide (carbohydrate) which naturally occurs in the human body. Exists in all animals and has the same structure in all species and theirs tissues. The concentration of HA is very high in the skin and the connective tissues. It is present even in the capsule of Streptococcus. It forms an essential structural component in the matrix of tissues where the concentration of HA is lower. [2]

1.1 History

In 1934, Meyer and Palmer separated an unknown polysaccharide from the vitreous humor of bovine eyes and named it hyaluronic acid because it contained uronic acid and its origins were found in the lens. [2] Because hyaluronic acid does not occur as an acid in the physiological enviroment, but as a sodium salt or other salts, it had been recognised as a group of hyaluronans since 1996. Its effect on such diseases as osteoartrityde, rheumatoid arthritis and other xeroderma was described 50 years ago. In the 1960s, it was considered that HA could be used in medicine; however the first success of its use was published in the 1970s. At the beginning, hyaluronan was used as a treatment for the joints of saddle horses. Later HA started to be used in veterinary medicine. In the early 80s, hyaluronic acid was registered as Healon and HA began to be utilised in the eye surgery for the protection of cornea. [3]

1.2 Chemical structures

Hyaluronan is the general term designating the repetition of a linear disaccharide containing D-glucuronic acid (Fig.1) and N-acetyl-D-glucosamine, interconnected by $\beta-(1-3)$ and $\beta-(1-4)$ interglycosidic bonds [1].

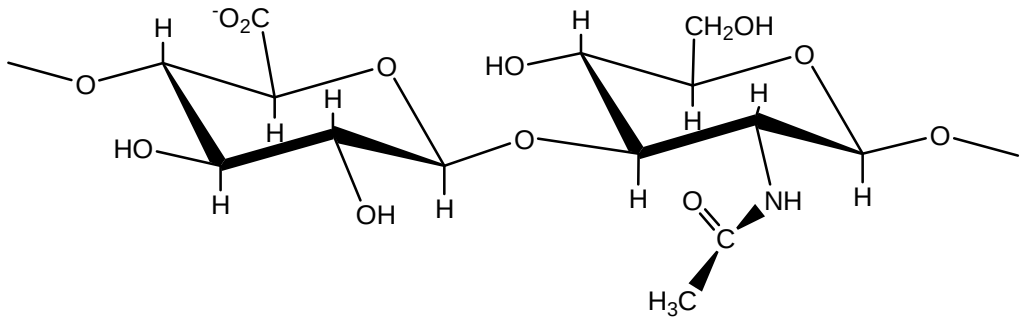

Figure 01 S*tructural unit of HA*

A hyaluronan molecule consists of glucuronic acid and N-acetylglucosamine units, which alternate over and over in the structure to form long chains. Each disaccharide unit has one carboxyl ate group, four hydroxyl groups, and an acetamido group. Hyaluronan differs from the other major glycosaminoglycans in that it does not contain sulphate groups. [4]

The chains can have the molecular weight of 4-5 MDa. Because the HA has hydrophilic and hydrophobic properties, it can create spiral configurations in water solutions. Hyaluronic acid works as a molecular „sieve"- it only goes over the small molecules such as water, but it can diffuse particles as electrolytes and nutriments. The most important property of hyaluronan is viscoelasticity. Viscoelasticity means that the substance with such property has the viscosity of liquids combined with the elasticity of some solids. Water solutions of HA are very viscous. Therefore, HA in joints decreases friction and absorbs the pressure impact.

1.3 Functions of Hyaluronan in medicine

Until the late 1970s, hyaluronan was described as a "goo" molecule, an ubiquitous carbohydrate polymer that comprised the extra cellular matrix. For example, hyaluronan is a major component of the extracellular matrix which forms synovial fluid. Along with lubricin, it is one of the fluid's main lubricating components. It helps to protect joints by increasing the viscosity of the fluid and by making the cartilage between bones more elastic. Furthermore, HA is a very important part of the physiology of skin, because it supports its function

and renovation. Another property of HA is the ability to bind water molecules and fill in the space between cells. Moreover, it helps the cells with their division and metabolic functions. In case there is a tumour growing or some disorder forming as a result of outer influences - such as exposition to UV radiation or the consumption of drugs - normal functions and convalescence could be lowered. Laboratory and clinical experiments show, that these changes are caused by lower concentration of free hyaluronan in the skin. HA keeps the structure of tissues and supports their defence. In addition, HA controls the transport of nutrients between cells and also the amount of water in the tissues. It creates suitable conditions for the division, migration and adhesion of cells. [5]

1.4 Degradation

Hyaluronan is degraded by a group of enzymes called hyaluronidases. In human body, there are at least seven types of hyaluronidase-like enzymes, several of which are tumour suppressors. The process of degradation of hyaluronan produces oligosaccharides and hyaluronans with a very low molecular weight. These products display pro-angiogenic properties. [5]

1.5 Medical applications

In 1950, HA was applicated in medicine, namely in the eye surgery as a vitreous replacement for the first time. Hyaluronan has been used as an effective medium in such branches of medicine as ophthalmic, orthopaedic or rheumatologic surgery. HA with high molecular weight (10 000 – 10 000 000 Da) is used as a cynosure in stomathology, mainly in the medical treatment for the affection of jowls. However, the major application of this material is in *ophthalmic surgery* due to its vicoelasticity. It is used during the implantation of intraocular lenses for people with cataracts [3]. It is also used as an inject able substance for degenerative-joint diseases.

Drug release is another promising application and utilisation of hyaluronan. Derivatives have been developed as topical, inject able, and implantable vehicles for the controlled and localized delivery of biologically active molecules. [6]

Hyaluronan is also used in hydrophilic coatings for a variety of medical devices, including catheters, guide wires and sensors to improve biocompatibility, lubricity, and to reduce fouling and tissue abrasion. By allowing devices to glide more smoothly it's easier for physicians to manipulate and steer devices around bends and through narrow passages in the body while minimizing trauma and tissue damage.

HA has also been successfully used in cosmetics. It has been extensively utilized in cosmetic products because of its viscoelastic properties and excellent biocompatibility. Application of cosmetic products containing HA to the skin is reported to moisture and restore elasticity effect, although there is no rigorous scientific proof this predicate. The combination HA with other polymer solutions or only pure acid hyaluronan is used in cosmetic surgery. [6]

2 STABILITY AND HOFMEISTER SERIE

At using the pure form of HA occurred a sequent degradation her structure. This degradation we can suppressed with an increment of ions (conserving substance), For example a benzalkonii chloridum, repressed this degradation and induce a denaturant. Disadvantage at using the conserving substance is happened to changes of HA. The changes we can reduce to increment of Hofmeister ions supported hydrophilic interaction HA and dissolved denaturant.

2.1 Hofmeister serie

The Hofmeister serie introduce a series of salts that have an influence on protein stability. *Hofmeister, Franz* **(1850-1922),** was an Austro-German chemist and one of the first protein scientist. He studied salts and their influence on the solubility and conformational stability of proteins. Hofmeister is known for his work on proteins, metabolism, and the chemistry of colloids. In 1888, he discovered the relative power of different anions which precipitate lyophilic salts. Hofmeister serie are not only fundamental to the theories about lyophilic colloids and their systems, but they are also important for physiology. In 1902, Hofmeister proclaimed that polypeptides were amino acids linked by peptide bonds. Interesting about it is that E. Fischer created the same model of primary protein structure at the same time, however, independently. [7]

2.1.1 Hofmeister interactions

Hofmeister discovered series of salts that have consistent effect on the solubility of proteins and on the stability of their secundary and tertiary structure .Hofmeister`s ion interactions affect and influnce the stability of proteins. Repeated questions about this topic are: „What is the mechanism of reactions of Hofmeister`s ions on proteins? “ and Why are some ions denaturants and some not?“ We can answer these questions by a model consisting of many studies, which reveal some characteristic points: [7]

1) They occur by the weak interaction models and each interaction can be characterised by a salting-out or salting-in constants.

2) The interactions are very specific and they define The Hofmeister series called also *lyotropic series*

3) The effect of the interaction is constantly changing, as definited by Setchenow equation

The Hofmeister serie named as „lyotropic series“ - an order of ability of ions to convert into salt-out proteins - have been known as a typical ion-specific phenomena of polymers in agueous system. A typical order for anions, respective cations is

$$(SCN^- \langle I^- \langle ClO_4^- \langle NO_3 \langle Br^- \langle ClO_3^- \langle Cl^- \langle BrO_3^- \langle F^- \langle SO_4^{2-}),$$

$$(K^+ \langle Na^+ \langle\langle Li^+ \approx Ca^{2+}).$$

But similar ion-specifications have been recently found in the swelling behaviour of several kinds of polymer gels [8]. Suzuki at all [9] investigated the ion effects on the thermal volume transition on a poly gel and he detected some kind of Hofmeister’s serie for the transition of temperature. He disagreed with the speculations that the origin of ion-effect is caused by the change in water structure around the hydrophobic group [8].

2.1.2 Mechanism of the Hofmeister serie

The mechanism of the Hofmeister serie seems to apply mainly to experiments on the solvent with higher salt concentration ($\rangle 100 mM$). Initial compounds in the series increase the solvent surface tension and decrease the solubility of non-polar „salt-out“ molecules, in effect, they strengthen the hydrophobic interaction. By contrast, final salts in Hofmeister serie increase the solubility of non-polar „salt-in“ molecules and increase the order in water, in effect, they weaken the hydrophobic effect. These salts interact directly with proteins and may even bind specifically. Ions have a strong „salting-in“ effect (example $I^- and\ SCN^-$ are strong denaturants, they salt in the peptide group and interact much more strongly with the unfolded form of protein than with his native form [7].

3 DIFFUSION

The one way as we can characterize dissolving process is monitoring the process diffusion dissolved HA by ions from Hofmeister serie. Diffusion is a spontaneous process characterizing a buffering of physical-chemical factors such as concentration press and temperature. The solute spontaneous to vibrated from the places with higher concentration into the places with lower concentration as long as the concentrations will be same. Diffusion may be used through over membrane which the definite component let transmit too. The diffusion process is some from way transmission masses at which the elements of masses such as atoms (ions, molecules) and vacancy moved to nearby elements. Because principle of diffusion is a thermal movement of the elements of masses so intensity the diffusion process is temperature dependent.

3.1 Fick's laws

Diffusion in solution it's controlled by the Fick's laws [10]. The first Fick's law say, that the diffusion flow is definated as materials of quantity of diffuse components *i* pass throw a unit area which is upright on the way diffusion. Diffusion flow is comparable to velocity addition:

$$J_i^{dif} = -D_i \cdot \frac{\partial c_i}{\partial x}, \tag{1}$$

where D_i is diffusion coefficient depended on characteristics of diffusing particles and background. The concentration gradient $\frac{\partial c_i}{\partial x_i}$ is concentration change with distance. In the arbitrary point at stationary diffusion is constant with the time.

The second Fick's law describe non-stationary diffusion, where concentration gradient isn't constant with the time and diffusion flow is changed with position [11]

$$\frac{\partial J_i^{dif}}{\partial x} = -\frac{\partial c_i}{\partial \tau} \quad \Rightarrow \quad \frac{\partial c_i}{\partial \tau} = D_i \cdot \frac{\partial^2 c_i}{\partial x^2}, \tag{2}$$

where $-\frac{\partial c_i}{\partial \tau}$ is speed change with time.

Diffusion coefficient describe a rate of move the dissolved components or gas. It's introduced an amount of components, which diffuse by the area about $1cm^2$ at the difference concentration $1g$ for distance $1cm$ during unit of time. The most useful methods of measuring the diffusion process are diaphragm cell, infinitive couple and Taylor dispersion. [11]

3.2 Non-Fickian diffusion

It is a specifics process, sometimes occurs at solutions of polymers by a good solvent. Actually, process of diffusion in this case no-proceed with Fick's laws. In contrast to Fick's laws the speed of the solvent penetrates into a polymer may not be proportional to the square root of time. It is result from configurationally changes in the polymer. The polymer molecules relax from their greatly configuration. The diffusion process can be faster than the relaxing process. The overall dissolutions is controlled by the relaxation kinetics, not Fick's laws. [12]

3.3 Polymer dissolutions

The polymer dissolution is important area of the interest in polymer-science and engineering because, dissolution is applicated, for example in the plastic recycling and drug delivery.[13] The polymers are dissolved very slowly because theirs dissolutions is controlled by diffusion of chains through a boundary layer adjacent to the polymer solvent interface. If the uncross linked and amorphous polymer will be in contact to solvent, so the solvent will be diffuse into the polymer and gel-like swollen layer is formed between polymer and gel-layer and gel-layer and solvent.

On the other hand, the crystallise polymer materials are dissolution instaneously, because the dissolutions process is direct by external mass transfer resistance through a liquid layer adjacent to the solid-liquid interface.

II. EXPERIMENTAL PART

4 MATERIALS AND METHODS

4.1 Used materials and preparation of samples

4.1.1 Used materials

- for this measurement were used following chemicals :
    - hyaluronan, rank 150806-D1/Contiprogroup
    - potassium iodine - **KI** (Riedel-da Haën Assay >99,5%)
    - potassium fluoride - **KF** (Fluka Assay >99,5%)
    - sodium chloride - **NaCl (**Fluka Assay >99,5%)
    - ethanol - **Ethanol** (Riel-de Haën)
    - distilled water - **H_2O**
    - magnesium chloride - **$MgCl_2$** (Fluka Assay >99,5%)

4.1.2 Preparations of samples

For the measurement were used solutions of anions with ionic strength $I = 0{,}1\,\text{mol/l}$ and for the preparation of polymer films of hyaluronan (molecular mass 630 kDa) were used. HA was dissolved in the water, then it was let swell and stirred for at least 24 hours at temperature (22-25°C). From the prepared solution the thin wedge films (0°10`<φ<1°) were boil off with. These films were drie in oast for 24h at 25°C. The prepared samples of hyaluronan were cut off from these films and as thin plates of rectangular cross-section having the area of $20 \cdot 10^{-6}\,m$ and the width of $8 \cdot 10^{-5} - 6 \cdot 10^{-6}\,m$. They were put in a temperature - controlled holder between two semitransparent glasses. The samples were so pressed by means of torsion sprinte that the density of lines and thus the internal pressure was the same for each measurement.

4.2 Methods

The time was always measured from the moment when the thermostatted solution was injected. The measurements were gauged at these temperature 25°C; 30°C; 35°C; 40°C; 45°C. In each measurement, 3-5 shots were taken at suitable time intervals. The resulting interferograms were scanned by digital camera *nikon coolpix 4500*. The example of interferogram can be seen in Figure 2.

Figure 1 *The example of interferogram ilustrated diffusion proces sof swelling (solid film of HA in KI solution at 35°C)*

4.2.1 Measurement of diffusion by wedge interferometry

These problems are very well described in the work of J. L. Duda. [14] He focused on some interference methods, which have been developed for the measurement of refractive index distribution that is associated with free-diffusion experiments. Optical configurations, the actual free diffusion takes place in a cell which to a large degrese independent on the particular Metod used to determine the concentration distribution. But traditional methods have many limitations. One of this limitation is that these methods are inefficient for the measurement of a small, strongly concentrated dependent diffusivity represented by

concentrated polymer solutions. But this limitation resolved a new interferometric technique recently, based on the formation of interference patterns by a thin wedge.

This technique makes it possible to work with relatively simple experimental apparatus and does not require accurate temperature control since the diffusion takes place in a thin channel in which natural convection currents are strongly suppressed. The interference patterns are photographed at different time intervals. These interferograms must be analyzed to obtain information concerning the distribution of refractive index at diffusion field. The fringes are contours of constants of constant optical layer. Consequently any increase of refractive index along a fringe must be evoked a decrease in the layer of the wedge.

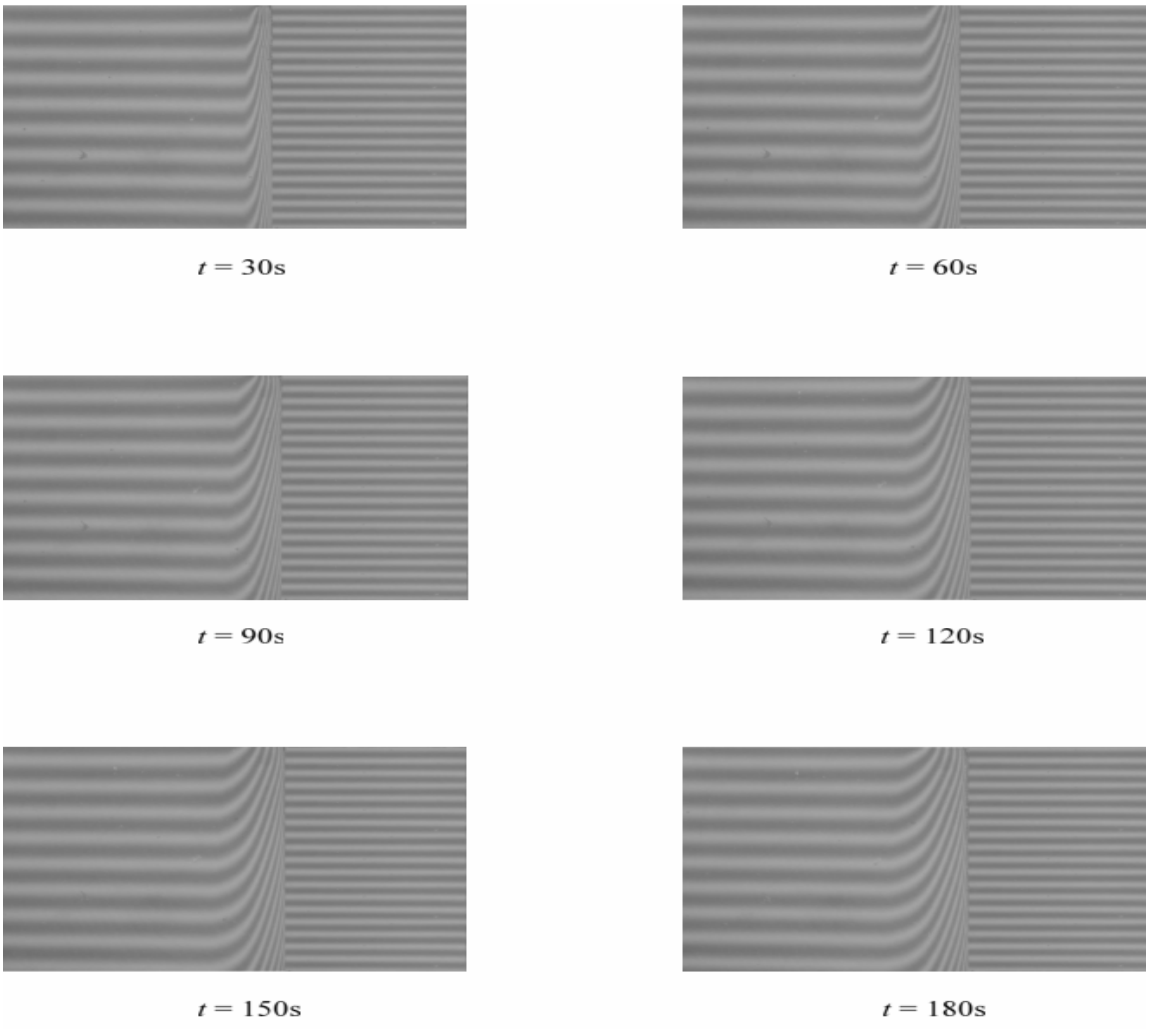

Figure 2 *Interferograms ilustrated diffusion process of swelling measurement at different times*

4.2.2 Microinterferometric method

The interferometric method enables us to determine the shape of concentration field in swollen surface layer (SSL) by two experimental procedures, *i.e.* the method of constant optical width or the method of constant width of wedge. The technique of constant width of wedge was also applied in this study and its principle is represented in (3) A straight line coalescing with parallel sections in pure polymer and solvent is drawn through the diffusion field obtained.

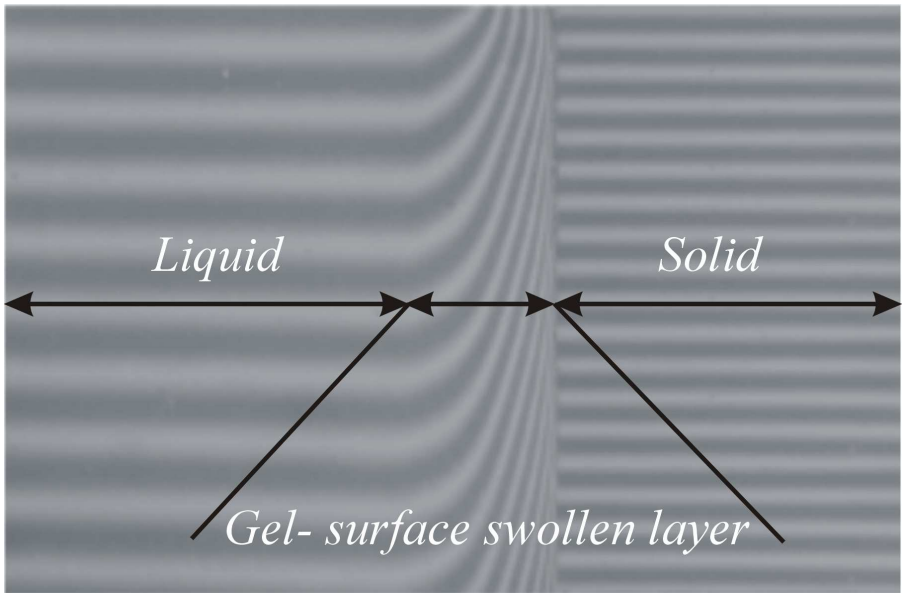

Figure 3 *Interferogram*

The interference fringes arise if the following equation is fulfilled as follows:

$$m\lambda = 2d(n_p - n_s), \tag{3}$$

where m, λ, d, n_p and n_s are an integer, wavelength, width of wedge, refractive index of pure solvent, and refractive index of polymer, respectively.

The condition need not be always fulfilled. The coalescence of parallel reference lines in polymer with interference lines in solvent is caused by perfect optical isotropy of particular regions of the swollen surface layer.

If K is the number of lines which intersect the reference line d = const., then the following equation is valid for the refractive index in the i-th point of intersection in ps-region:

$$n_{ps,i} = n_p + i\frac{n_s - n_p}{K} \tag{4}$$

By measuring the position of the points of intersection in relative scales (with respect to magnification) and using equation (4), the curve $n = f(x)$ may be constructed. The accuracy of the n-c transformation depends on the number of lines intersecting the reference line (number of lines = $f(n_p - n_s)$) and the distance between semi-transparent glass plates.

The wedge microinterferometer may be used only if the refractive index of solvent n_s is different from refractive index of polymer n_p. In this case, two spots with different concentrations have different indices of refraction. The change in refractive index is proportional to concentration change in a not too wide concentration interval.

The mutual diffusion in a system polymer-solvent with one-dimensional experimental arrangement and stationary flow may be described by the second Fourier-Fick law (system with concentration dependent diffusion coefficient) in the form:

$$\frac{\partial c}{\partial t} = \frac{\partial}{\partial x} D \frac{\partial c}{\partial x}, \tag{5}$$

where c is concentration of polymer expressed arbitrarily.

The solution for the following initial and boundary conditions:

$$\begin{aligned} c &= c_0 \quad x \rhd 0 \quad t = 0 \\ c &= 0 \quad x \lhd 0 \quad t = 0 \\ c &= c_0 \quad x \to \infty \quad t \rhd 0 \end{aligned}$$

ensues from the Boltzmann solution of partial differential equation (5) and its mathematical application put forward by Matano.

Provided the mixing of both components is not accompanied by volume change, the equation allowing to calculating the diffusion coefficient may be written in the form:

$$D = \frac{1}{\rho}\frac{\mathrm{d}\eta}{\mathrm{d}\omega_1}\left(\int_{\rho_{10}}^{\rho_1} 2\eta d\rho_1 + \omega_1 \int_{\rho_0}^{\rho} 2\eta d\rho\right) \tag{6}$$

where D is diffusion coefficient, ρ and ω_1 are total solution density and mass fraction, ρ_0 and ρ_{10} are total density and species density at the limit of the diffusion field, $\eta = x/2t^{1/2}$ is a variable defined such that x is the distance from the initial boundary at which contact occurs and t is the time which passed since the two solutions mde their initial contact. [16]

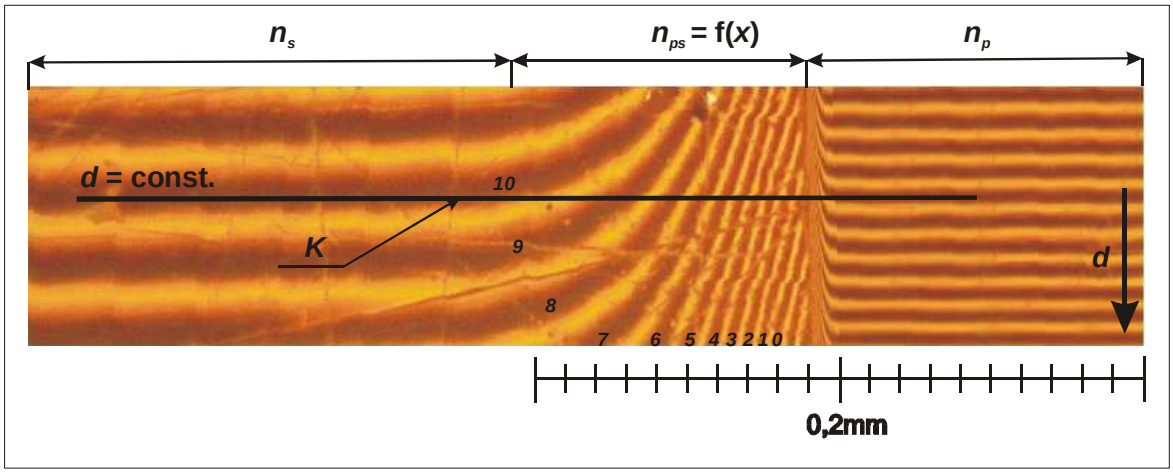

Figure 4 *Interferogram obtained by the technique of constant wedge width and its interpretation (sample of Hyaluronan at 37°C in t=210 seconds); n_s – refraction index of pure solvent; n_p – refraction index of polymer; n_{ps} – refractive index in SSL; 0, 1, 2, …,K – number of crossed lines; d – width of wedge.*

A microinterferometer based on light interference in a thin wedge layer formed between two semi-transparent walls was used for the measurements. The method is very suited for the investigation of diffusion because it makes it possible to shorten the time of experiments by several times and reduces the exigencies of thermostatting, owing to the diffusion taking place in relatively viscous medium.

The films were prepared from the biopolymers (assigned in Experimental part, section A. Materials) by pouring the solution in redistilled water in a Petri dish. The resulting interferograms were scanned by a digital camera **NIKON COOLPIX 4500**.

For image analyses of interferograms and the computing of $c = f(x)$ relation was used **SAIA** software [15] developed by P. Urban (Institute of Physics and Materials Engineering, FT, UTB in Zlin). [16,17]

5 RESULTS AND DISCUSSION

Our objective was to find what influence do ions of Hofmeister series have on the swelling process by hyaluronan. These ions had a great influence on tertiary and quaternary structure of proteins and on the stability of proteins. Hyaluronan is a polysaccharide which has a relatively large molecular mass. The molecular mass of HA causes a nodus of all chain molecules and an enlargement of the supramolecular structures, which consequently created different interactions in the final coil. Iont which promote water-repellent and water-receptive interactions have been found, which can influence the whole diffusion process of swelling.

The influence of Hofmeister series on HA behaviour and process of swelling Hyaluronan films we can measure by interferometric method. The diffusion process of swelling measurement is shown in Figures The results of the apparent mutual diffusion coefficients D_s of solvents into HA thin films corresponds with viscosity values, where is the influence of Hofmeister interaction evidently more visible.

The influence of Hofmeister series ions on hyaluronan behaviour and hyaluronan films swelling by solutions of these ions can be studied by interferometric methods. The dependence of the mutual diffusion coefficient of swelling on the ability of water to solvate hyaluronan was verified. As can be seen in Figure 5, the relation between the mutual diffusion coefficient and hyaluronan concentration in swollen layer shows a statistically significant decreasing rate of swelling diffusion process for experiment with *KF* addition (Ionic strength I = 0,1 mol.L^{-1}) as compared with swelling of hyaluronan by pure water. On the other hand, solutions of *NaCl*, $MgCl_2$ and *KI* force diffusion swelling processes.

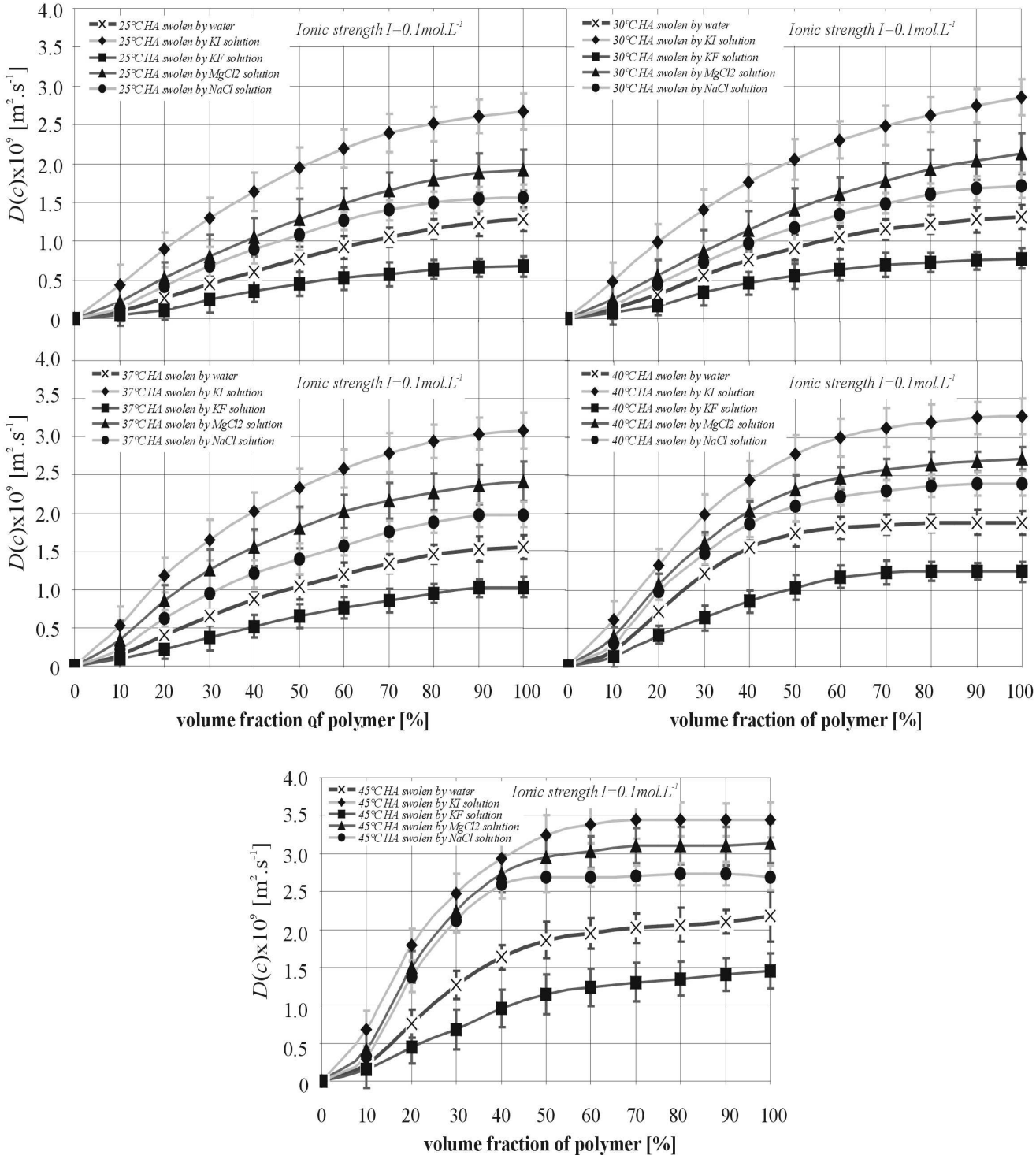

Figure 5 *Relation between mutual diffusion coefficient and HA concentration in surface swollen layers.*

Differences between monovalent cations (Na^+, K^+) and divalent cations (Mg^{2+}) at ionic strength I = 0,1 mol.L^{-1} are consistent with predictions that charge shielding by divalent ions is greater [8, 9]. The data for the swelling diffusion process suggests that Mg^{2+} causes greater domain contraction, which is additional to the electrostatically induced changes in HA properties observed with K^+ and Na^+. Individual Mg^{2+} ions may co-ordinate two carboxy groups on the same HA chain secondary structure, and promote chain contraction. Consequently, cations of Mg^{2+} could induce a significant reduction of chain stiffness. However, the mutual diffusion coefficient $D(c)$ of swelling shows (Figure 6) nearly order of magnitude differences for *KI* solutions in comparison with *KF* ones. In this case, the influence of the anions (F^-, Cl^-, I^-) on the behaviour of the K^+ cation is evident. This fact can be viewed as in accordance with theory of Hofmeister ion series (hydrophilic→ I^{-1}<Cl^{-1}<F^{-1}←hydrophobic). F^- or Cl^- ions may alter the co-ordination of water molecules with HA chains, thereby disrupting the hydrogen bonds involved in water bridges. The strong reduction of hydrophilic chain interaction and creation of hairpin loops caused by F^- can consequently induce a significant increase of chain stiffness. The lower ionic strength shows similar dependences (see in Figure 6), which are represented by the mean mutual diffusion coefficient.

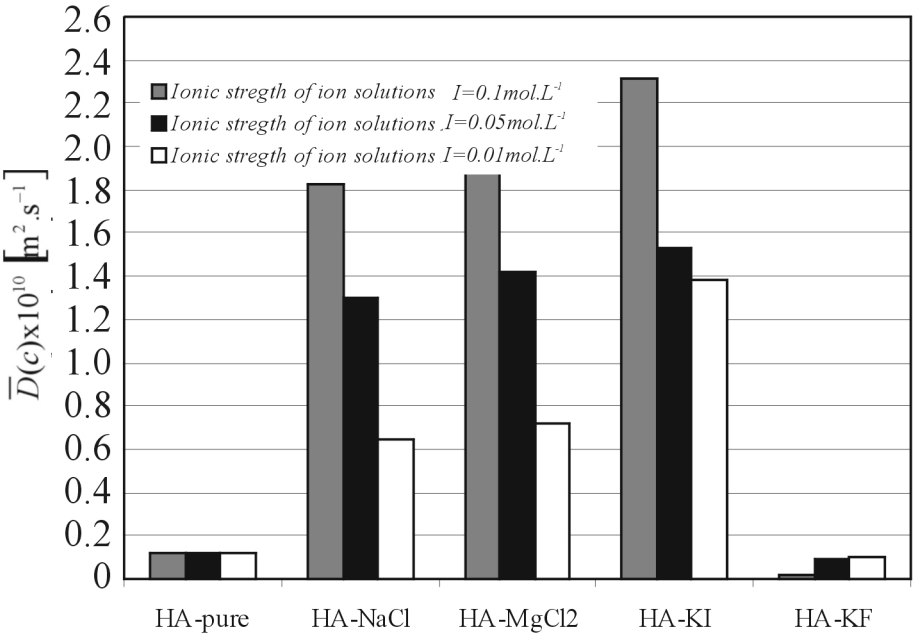

Figure 6 *The mean mutual diffusion coefficients of HA solutions*

CONCLUSION

The results of the apparent mutual diffusion coefficients D_s of solvents into HA thin films corresponds with viscosity values, where is the influence of Hoffmeister interaction evident. These results of presented experiment relatively support the theory of Hoffmeister series. The assumption of increasing or weakening hydrophobic interaction by assorted ions probably can be used in connection with behaviour of Hyaluronan in solutions. But for these measurements would be make other experiments for different values ionic strength. These experiments should be much distorted.

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LIST OF SYMBOLS AND ABBREVIATIONS

HA	Hyaluronan acid
KCl	Potassium chloride
$CaCl_2$	Calcium chloride
$MgCl_2$	Magnesium chloride
$ZnCl_2$	Zinc chloride
KBr	Potassium bromide
KI	Potassium iodine
I	ionic strength
D	Diffusion coefficients
J	Diffusion flow
c	concentration
φ	Angle of wedge
τ	Time diffusion

LIST OF PICTURE

APPENDICES

APPENDIX P I:

Gřundělová, L., Pokopcová, A., Varhaníková, J., Mráček, A., Bilerová, H., Velebný, V.: The Influence of Hofmeister Ions Series on Hyaluronan Swelling and Viscosity. *Juniormat 07*, 19.-20. September 2007, Brno (Czech Republic).

APPENDIX P II:

Mracek A., Varhanikova J., Lehocky M., **Grundelova L.**, Pokopcova A., Velebny V., The Influence of Hofmeister Series Ions on Hyaluronan Swelling and Viscosity. *Molecules* (13) 2008 1025-1034 DOI: 10.3390/MOLECULES13051025

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