

The diffusion process of sodium hyaluronate (Na-HA) and Na-HA-*n*-alkyl derivatives films swelling

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Abstract: The process of diffusion of sodium hyaluronate and its hydrophobically modified alkyl derivatives in water was studied by measuring the kinetics of solid polymer film swelling. The following fundamental thermodynamic parameters of the swelling process were calculated: the apparent diffusion coefficient of swelling by water D_s , determined at three temperatures (25, 37, and 45°C), the activation enthalpy of diffusion connected with the swelling $\Delta H_{D,s}$ and the activation enthalpy of the swelling process ΔH_s . The thermodynamic activity of the solvent

for the given sodium hyaluronate and its four alkyl derivatives has been expressed through the internal quantity $RA_{\delta,s}$, that is expansion work of polymer coil accomplished by the action of the internal pressure. © 2007 Wiley Periodicals, Inc. *J Biomed Mater Res* 83A: 184–190, 2007

Key words: hyaluronan; HA-*n*-alkyl derivatives; hydrophobic modification; swelling; activation enthalpy; diffusion process of swelling; sodium hyaluronate

INTRODUCTION

Great importance of hyaluronic acid, also called hyaluronan (HA), discovered by Meyer and Palmer in 1934,¹ has been described many times. HA is a naturally occurring linear polymer, which belongs to a wide class of polysaccharides, called mucopolysaccharides or glycosaminoglycans. Its monomeric unit is composed of a repeating disaccharide containing D-glucuronic acid and N-acetyl-D-glucosamine, interconnected by β -(1–3) and β -(1–4) interglycosidic bonds (Fig. 1).

HA occurs everywhere in the living organisms, mainly in the form of a gel-like substance of the vitreous humor of the eye, a constituent of the synovial fluid of the joints and an extracellular matrix of all connective tissue. HA and other glycosaminoglycans are particularly interesting due to their possible role as cellular recognition sites. It is based on its physiological and physicochemical properties. Hyaluronic acid has distinctive passive and active biological functions. In the living organisms for example it participates in the influences regulation of cell growth, distribution of the plasma proteins, diffusion of other

macromolecular substances, and transport processes in the tissue. In the synovial fluids, HA behaves as a lubricant and muffs impacts by creating the specific bonds with other proteoglycans ensuring the stability of the higher order organized structures.

The molecular weights of HA polymers cover the range from hundreds of thousands to up to several millions Daltons, depending on the source and method of isolation² or on the type of microbial cultivation.^{3,4} With respect to its polyanionic character, HA is able to form a strong bond with alkaline metal or calcium cations. Its biological and physiological function depends on the viscoelasticity of HA solutions. It is a complex function of polymer concentration, ionic strength, pH of the solvent, and the shear rate. It seems, the authors show very strong dependence of viscosity on the rate gradient and frequency dependence of elasticity.^{5–8}

From the 1980th, HA has been applied in various clinical applications^{9,10} such as ocular and cardio surgery, viscosupplementation for arthritis,^{11,12} wound healing and plastic surgery, where HA has been generally used as an antiadhesive component. HA and its derivatives were already investigated as the vehicles for the local, injection, and implantation controlled drug delivery system.¹³ Nowadays, HA is one of the most important basic biopolymers for preparation of a new biocompatible and biodegradable materials for applications in cosmetics, pharmaceuticals, and medicals

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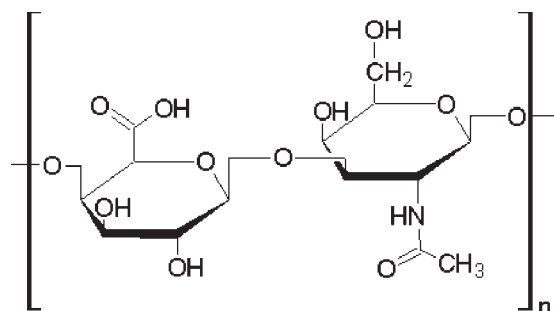


Figure 1. Structural unit of hyaluronic acid.

as well as in tissue engineering.^{14,15} Because of the high quality and the slight contents of proteins and microbial contaminants, HA synthesised by bacteria is currently being used.

The interest is also focused on the development of derivatives preserving the polymerization degree of the polymer, while maintaining biologically and physiologically important side chain groups—negatively charged carboxylic and acetamidic groups. For this reason, the new hydrophobically modified sodium hyaluronate (Na-HA) derivatives have been prepared (Fig. 2).

It is obvious that the behaviour of these new Na-HA-derivatives dissolved in physiological solvent is one of the crucial questions. Diffusion of low molar mass solvents into polymer films can be evaluated on the basis of parameters describing the surface diffusion layer formation. In some investigated cases it has been recognized that polymer matrix swells because of the solvent effect, the developed surface diffusion layer is denoted as a surface swollen layer (SSL).^{16,17} The technique based on interference monochromatic light was used for characterization of surface swollen layer formation of sodium hyaluronate and hydrophobically modified Na-HA-derivatives. The latter method, the so called “wedge interferometry,” was developed earlier by Lapčik and co-workers^{18–20} or Duda et al.²¹ Results of measurements on this apparatus were used for calculating the following thermodynamic and kinetic parameters: the apparent diffusion coefficient of swelling by water D_s determined at three temperatures (25, 37, 45°C), the activation enthalpy of diffusion connected with swelling $\Delta H_{D,s}$ and the activation enthalpy of swelling process ΔH_s . The thermodynamic activity of solvent for given Na-HA and its four alkyl derivatives has been expressed through the internal quantity $RA_{\delta,s}$, that is, expansion work of polymeric coil accomplished by action of the internal pressure.

THEORETICAL BACKGROUND

In all cases presented in this article, thickness of the swollen surface layer at constant temperature is proportional to $t^{1/2}$. This has been predicted by Crank²²

for boundaries resulting from a discontinuous change of diffusion constant with concentration. Considering the fact, that microscopic methods (i.e. interferometric method) allow the determination of the thickness of the swollen surface layer δ and velocity of penetration \dot{s} in one experiment. It is suitable to obtain function describing the time dependence of the thickness $\delta = \delta(t)$ of the swollen surface layer. This may be derived by solving the second Fick's law equation

$$\frac{\partial c}{\partial t} = D_s \frac{\partial^2 c}{\partial x^2} \quad (1)$$

with following boundary conditions:

$$\begin{aligned} t = 0; \quad c = 0 \quad \text{if } x > 0, \quad \text{and } c = c_0 \quad \text{if } x = 0, \\ t = t; \quad c = c_0 \quad \text{if } x = 0, \quad \text{and } c \neq 0 \quad \text{if } x > 0. \end{aligned}$$

The Eq. (1) can be solved by application of Laplace's transformation and results in Refs. 23–25 and 22:

$$\delta = \sqrt{2D_s t} \Rightarrow D_s = \delta^2/2t, \quad (2)$$

where δ is thickness of swollen surface layer.

The solvent molecules do not only penetrate into the polymer but also swell it. Thus, the boundary between the liquid and the swollen polymer also changes with time, a factor not dealt with when applying Crank's treatment. This difficulty can be solved by using a moving coordinate system where the reference plane is the outer boundary of the swollen surface with the solvent concentration at the surface. The quantity D_s in Eq. (2) can be understood as apparent diffusion coefficient for the low concentration region of solvent in the swollen surface layer where the concentration dependence of D_s might be neglected.

The dissolution measurements indicate that D_s is not dependent upon the molar mass over a large range of chain lengths of the linear macromolecules.¹⁷ However, the temperature dependence of D_s is evident and can be described by¹⁷:

$$D_s = D_0 \exp\left(-\frac{\Delta H_{D,s}}{RT}\right), \quad (3)$$

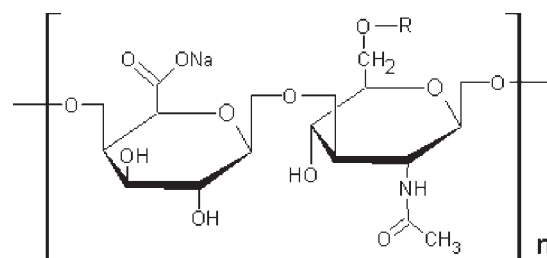


Figure 2. Structural unit of sodium hyaluronate derivatives (R-alkyl chain).

where D_0 is the preexponential factor, $\Delta H_{D,s}$ is the activation enthalpy of diffusion connected with swelling under the constant pressure, R is the universal gas constant, T is the absolute temperature.

Many years ago, Überreiter and Asmussen discovered that the maximum concentration gradient could not be located in front of the diffuse boundary, but only in the certain point (which should be very close) behind this region. For this reason the optical interface movement is not expected to be exactly identical to the movement of the front line of the diffusion boundary. The influence of this factor can be determined during the analysis of the empirical parameter A_δ , which appears in the equation for the temperature dependence of the surface layer thickness in the following analogous formula to the Eq. (3)^{16,17}:

$$\delta = \delta_0 \exp\left(-\frac{A_\delta}{T}\right), \quad (4)$$

where δ is the total thickness of the surface swollen layer, δ_0 is the preexponential factor, A_δ is the empirical parameter, and T is the absolute temperature.

Variables $\Delta H_{D,s}$ and A_δ can be calculated on the basis of interferometric measurements by using logarithmic form of Eqs. (3) and (4). Consequently, an equation for the activation enthalpy of swelling can be derived:

$$\Delta H_s = \Delta H_{D,S} - RA_{\delta,s}, \quad (5)$$

where $\Delta H_{D,s}$ is the activation enthalpy of diffusion connected with swelling under the constant pressure and $RA_{\delta,s}$ [product of gas constant and empirical constant A_δ calculated by using logarithmic form of Eq. (4)] was derived by Lapčík and Valko²⁰ and can be understood as the voluminous work accomplished by polymer coils being affected by internal pressure and associated with their transition from solid polymer phase into a swollen gel of the swollen surface layer.

EXPERIMENTAL

Materials

For the preparation of the hydrophobically modified Na-HA-derivatives, the alkylation procedure described by Lapčík,²⁶ have been used. Polysaccharide chain has been modified by substituting the hydrogen of the primary

hydroxyl group of the *N*-acetyl-D-glucosamin unit by linear alcohol with the different chain length. The degree of substitution was calculated for every 20th disaccharide subunit to 100% of the theoretical yield of the reaction. The amounts of added alcohols were based on the molar ratio of primary hydroxyl groups in HA to hydroxyl groups of the linear alcohol. Synthesis of the alkyl derivatives takes several days of preparation and was described in detail previously.^{27,28} Four different derivatives of sodium hyaluronate, with the different alkyl chain length (C8, C10, C14, and C16) were synthesized²⁸ and are shown in Figure 2.

All materials and chemicals used for the synthesis were of standard purity p.a. Sodium hyaluronate (M_w , 8.5×10^5 Da) was obtained from the Contipro, Czech Republic. Solvents (pyridine, dimethylsulphoxide, and ethanol, purity for UV spectroscopy) and molecular sieve were purchased from Fluka, calcium hydride, kalium hydroxide, and linear alcohols (octanol, decanol, tetradecanol, and hexadecanol, purity for UV spectroscopy) were obtained from Sigma-Aldrich. The process of the surface swollen layer formation was studied on the solid films of polymer samples prepared by casting from the 0.01M concentration solutions in redistilled water and by subsequent evaporation in dessicators. The redistilled water was used as solvent for the kinetics measuring of dissolution too.

Molar mass determination

The measurements were executed at 25°C using Ubbelohde dilution viscometer (Schott Geräte Mod. AVS/T2). Mark-Houwink equation in the conventional form $[\eta] = K'M_w^a$ was used for the regression analysis of the intrinsic viscosity ($[\eta]$) dependencing on the molar masses (M_w) of the Na-HA and its derivatives samples. This equation was fitted with K' and a denotes numerical values of 2.78×10^{-4} and 0.78, where K' and thus $[\eta]$ are in dL/g units.²⁸ Parameters of Mark-Houwink equation are summarized in the Table I.

The surface swollen layer thickness characterization

The direct measurement of the surface swollen layer thickness (SSL) was taken in the wedge interferometer described in previous works.¹⁸⁻²⁰ Experiments were performed at three temperatures (25, 37, and 45°C). The thickness of SSL in definite time was measured from recorded interferograms. A typical interferogram can be seen in Figure 3, which illustrates concentration field of Na-HA in the surface swollen layer expressed as the change of

TABLE I
Parameters of Mark-Houwink Equation

Parameter	Samples				
	Na-HA	Na-HA-C8	Na-HA-C10	Na-HA-C14	Na-HA-C16
M_w (Da)	8.50×10^5	1.03×10^5	1.41×10^5	2.40×10^5	3.82×10^5
$[\eta]$ (dL g ⁻¹)	15.51	7.34	3.6	5.36	7.93

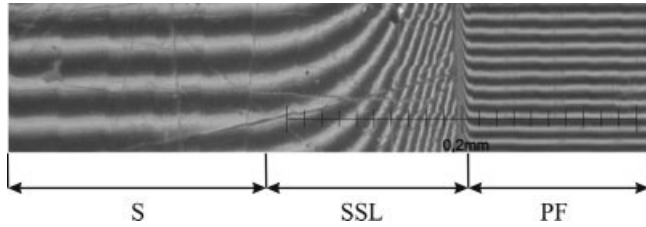


Figure 3. Interferogram obtained by measuring of unmodified sodium hyaluronate (temperature, 37°C; swelling time $t = 160$ s), S, solvent (H₂O); SSL, surface swollen layer; PF, polymer film.

refractive index. The microinterferometer used for measuring is based on light interference in a thin wedge layer formed between two semitransparent walls, which can be found in Refs. 29 and 30. This method is very suitable for the investigation of diffusion because it enables to shorten the time of experiments and reduces the exigencies of thermostating, owing to the diffusion taking place in relatively viscous medium. The diagram of this instrument and its principle is in Figure 4.

Samples were cut off these films and so that the thin plates of rectangular cross-section had the area of $(10 \times 2) \times 10^{-6}$ m² and the width of 8×10^{-5} to 6×10^{-4} m. They were put in a temperature-controlled holder of the volume of $(23 \times 20 \times 3.5) \times 10^{-9}$ m³ between two semitransparent glass plates with the transparence of about 40%. The time was always measured from the moment when the thermostated solution was injected. The temperature was held exactly constant with deviation $\pm 0.1^\circ\text{C}$. In each measurement, 3–5 shots were taken at convenient time intervals. The resulting interferograms were scanned by digital camera NIKON COOLPIX 4500.

SAIA software³¹ developed by P. Urban (Department of Physics and Materials Engineering, FT, TBU in Zlin) was used for the image analyses of interferograms relation.

Statistical data processing

The five measurements were evaluated for each film of Na-HA (and its derivatives) and the apparent diffusion coefficients of swelling by water and the thickness of surface swollen layer at each temperature were computed from each measurement. The mean value and relevant standard error of estimate for confidence interval at appropriate significance level were computed for measured values at given temperature. The other magnitudes ($RA_{\delta,s}$, $\Delta H_{D,s}$, ΔH_s) were calculated by using logarithmic form of Eqs. (3) and (4) formulas and only three temperature values were used for the formula regression, because the number of samples was restricted. Consequently, standard errors of estimate for these magnitudes, $RA_{\delta,s}$, $\Delta H_{D,s}$, ΔH_s , were computed for confidence interval at 80% significance level.

RESULTS AND DISCUSSION

The apparent diffusion coefficients of swelling by water (D_s) given in Table II were calculated from the line slope of the time dependence $\delta^2(t)$ (see example in Fig. 5) in accordance with Eq. (2). Molar masses of unmodified and modified Na-HA were calculated from Mark–Houwink equations obtained by viscosity measurements, withal the values have not been differed in order between individual variants. As can be seen, values of the apparent diffusion coefficients of swelling by water (D_s) increased with rising temperature for all samples which is in agreement with the logical presupposition of Na-Ha-*n*-alkyl derivatives hydrophobic character. However, in the case of alkyl derivatives of Na-HA a decrease of D_s was obtained. In other words, longer alkyl chain can logically con-

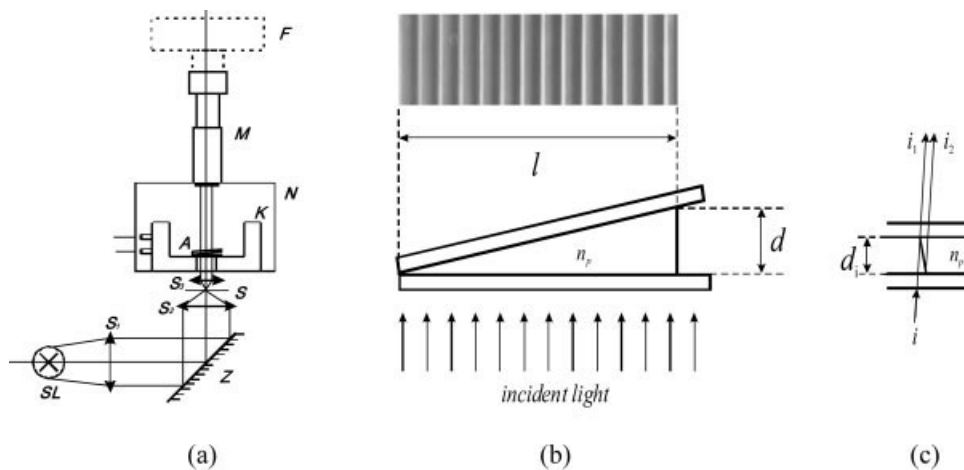


Figure 4. (a) Diagram of interferometer used. SL, sodium-vapour lamp; S1-S3, lenses; Z, mirror; S, slit; K, temperature-controlled vessel; N, base of microscope; M, microscope; F, CCD camera; A, holder of glass plates with sample; (b), (c) Principle of interferometric method. n_p , refractive index of studied polymer; d , thickness of wedge; d_i , thickness of wedge in i -th place; i , light beam; i_1 , i_2 , interfering beams.

TABLE II
The Apparent Diffusion Coefficients of Swelling by Water Na-HA and Na-HA-*n*-Alkyl Derivatives at Three Different Temperatures

Sample	M_w (Da)	$D_{s,25^\circ\text{C}}$ ($\text{m}^2 \text{s}^{-1}$)	SEE ($D_{s,25^\circ\text{C}}$) ($\text{m}^2 \text{s}^{-1}$)	$D_{s,37^\circ\text{C}}$ ($\text{m}^2 \text{s}^{-1}$)	SEE ($D_{s,37^\circ\text{C}}$) ($\text{m}^2 \text{s}^{-1}$)	$D_{s,45^\circ\text{C}}$ ($\text{m}^2 \text{s}^{-1}$)	SEE ($D_{s,45^\circ\text{C}}$) ($\text{m}^2 \text{s}^{-1}$)
Na-HA	8.50×10^5	10.1×10^{-12}	0.4×10^{-12}	11.7×10^{-12}	0.5×10^{-12}	13.0×10^{-12}	0.7×10^{-12}
Na-HA-C8	1.03×10^5	5.0×10^{-12}	0.6×10^{-12}	6.7×10^{-12}	0.6×10^{-12}	10.1×10^{-12}	0.3×10^{-12}
Na-HA-C10	1.41×10^5	3.3×10^{-12}	0.8×10^{-12}	5.0×10^{-12}	0.8×10^{-12}	8.3×10^{-12}	0.7×10^{-12}
Na-HA -C14	2.40×10^5	1.7×10^{-12}	0.5×10^{-12}	3.3×10^{-12}	0.5×10^{-12}	5.0×10^{-12}	0.7×10^{-12}
Na-HA -C16	3.82×10^5	1.0×10^{-12}	0.3×10^{-12}	1.7×10^{-12}	0.3×10^{-12}	3.3×10^{-12}	0.6×10^{-12}

M_w , molar mass; $D_{s,25^\circ\text{C}}$, $D_{s,37^\circ\text{C}}$, $D_{s,45^\circ\text{C}}$, the apparent diffusion coefficients of swelling by water at three different temperature; SEE, standard error of estimate for confidence interval at significance level 95%.

tribute to a lower D_s , because hydrophobic character of alkyl chains is more manifested. These qualitative results of the apparent diffusion coefficients of swelling by water were presupposed and quantitative evaluation can be seen in Table II. Interesting information about influence of alkyl chains and temperatures can be seen in Figure 6. It has been found that Na-HA-C8 and Na-HA-C10 show a higher D_s with increasing temperature than Na-HA, Na-HA-C14, and Na-HA-C16. This fact leads to the assumption that increased mobility of molecular segments of alkyl derivatives of Na-HA caused by higher temperature eases diffusion of water in the case of shorter alkyl chains. On the contrary, as for as longer alkyl chains in Na-HA-*n*-alkyl derivatives are concerned, this effect was not observed.

The values of activation parameters describing the diffusion process of polymer swelling under study are shown in Table III. These values are considerably influenced by the polarity of the basic structural polymer unit—a monomer. Modern studies report on the important role of hydrogen bonds in the hyaluronate chain structures^{32–35} and some authors associate problematic of hyaluronate swelling with intermolecular entanglement caused by hydrogen bonds.^{36,37} From the physical point of view, hyaluronic acid

functionality can mostly be traced back to the swelling properties of polyelectrolyte networks. The molecule of hyaluronan behaves in water solutions like a negatively charged polyelectrolyte and its size, shape, conformation, and its degree of hydration depends on the degree of dissociation which is determined by pH.³⁶ In the diffusion process of swelling compatibility and the affinity between the polymer film and the solvent are very important, because the solvent molecules must penetrate into the polymer and than the polymer chain molecules must be solvated by solvent molecules. Subsequently, the diffusion process of swelling continuous with the expansion of polymer coils and its aggregates, increase of polymer mobility, and permeability.³⁸ From this point of view, it is highly suitable to use pure water as solvent for hyaluronan film swelling. On the other hand, alkyl chains in Na-HA-*n*-alkyl derivatives are hydrophobic character. Consequently, as can be seen in Figure 7, the voluminous expansion work $RA_{\delta,s}$ for Na-HA is supposed to be relatively higher than for Na-HA derivatives. As it is evident from Table III, the values of activation enthalpy of diffusion connected with swelling $\Delta H_{D,s}$ and activation enthalpy of swelling ΔH_s

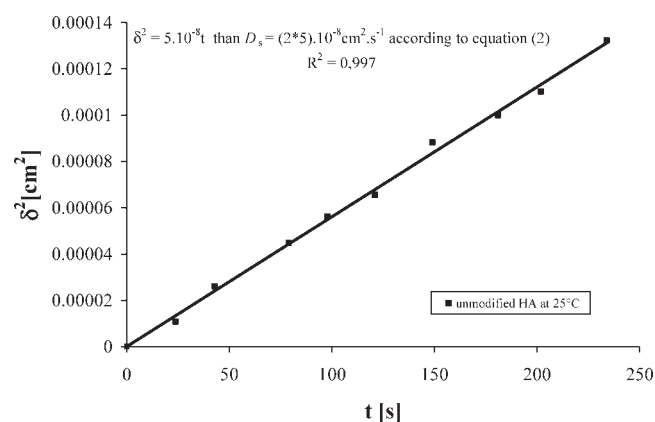


Figure 5. The example of D_s determination from the line slope of the time dependence $\delta^2(t)$.

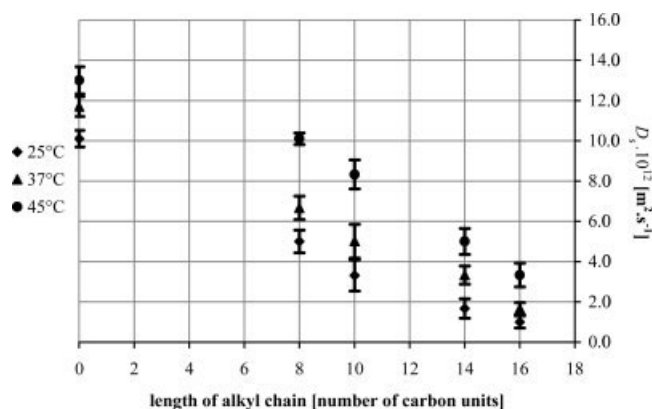


Figure 6. Relation between the apparent diffusion coefficient of swelling by water and the alkyl chain length of Na-HA in water (diamonds, $T = 25^\circ\text{C}$; triangles, $T = 37^\circ\text{C}$; circles, $T = 45^\circ\text{C}$).

TABLE III
Swelling Activation Parameters of Na-HA and Na-HA-*n*-Alkyl Derivatives Under Constant Pressure

Sample	$RA_{\delta,s}$ (kJ mol ⁻¹)	SEE ($RA_{\delta,s}$) (kJ mol ⁻¹)	$\Delta H_{D,s}$ (kJ mol ⁻¹)	SEE ($\Delta H_{D,s}$) (kJ mol ⁻¹)	ΔH_s (kJ mol ⁻¹)	SEE (ΔH_s) (kJ mol ⁻¹)	ΔH_d (kJ mol ⁻¹)	SEE (ΔH_d) (kJ mol ⁻¹)
Na-HA	8.4	0.3	10	1	2	1	4.4	0.8
Na-HA-C8	9.8	0.4	27	1	17	2	21	1
Na-HA-C10	10.2	0.3	35.0	0.5	25	1	30	2
Na-HA-C14	15.2	0.4	43.0	0.6	28	1	33	3
Na-HA-C16	16.9	0.2	47.5	0.4	30.4	0.8	35.0	0.9

$RA_{\delta,s}$, the voluminous work accomplished by polymer coils affected by internal pressure and associated with their transition from solid polymer phase into a swollen gel of the swollen surface layer; $\Delta H_{D,s}$, the activation enthalpy of diffusion connected with swelling; ΔH_s , the activation enthalpy of swelling; ΔH_d , the activation enthalpy of dissolution; SEE, standard error of estimate for confidence interval at significance level 80%.

are largely dependent upon the length of substituted alkyl chain and longer chain increases these values. On the other hand, in the case of shorter segment alkyl chains, the flexibility of Na-HA¹⁴ can lead to lower values of $\Delta H_{D,s}$ or ΔH_s . As can be seen in Figure 8, activation enthalpy was strongly increased in Na-HA-C8 compared with Na-HA and a similar increase was observed between Na-HA-C8 and Na-HA-C10, whereas differences in values of ΔH_s between Na-HA-C12 and Na-HA-C16 have not been so significant. It is assumed, that the solvation of Na-HA and its alkyl derivatives results in the decreasing flexibility of the macromolecular coil and results in the rise of the potential barrier, which must be overcome during the diffusion process of swelling which can be distinguished from interferogram.^{21,39}

The values of activation parameters depend upon molar mass (M_w) and they increase with the growing value of M_w , which may be ascribed to the mutual chain entanglement caused mainly by the higher number of side chain structures in the higher molar mass fractions. The fact that molar masses of Na-HA-*n*-alkyl derivatives have been similar to unmodified

Na-HA (Table II), the activation parameters ΔH_d (the activation enthalpy of dissolution) and ΔH_s (the activation enthalpy of swelling) of Na-HA-*n*-alkyl derivatives have been increased contrary to Na-HA. This seems to support the presupposition of Na-HA-*n*-alkyl derivatives hydrophobic character.

CONCLUSION

The effect of Na-HA macromolecular chain modification by alkylation with linear alkyl chains on diffusion of low molar mass solvents (in our case water) at three different temperatures was studied. Obtained results were close to theoretical predictions, where hydrophobically modified polymer shows a decrease of the apparent diffusion coefficient of swelling by water value with increasing alkyl chain length. Modification by alkylation caused retardation of swelling and decrease of dissolution velocity of studied polymer films. From the formal thermodynamics point of view of swelling and dissolution has been strongly

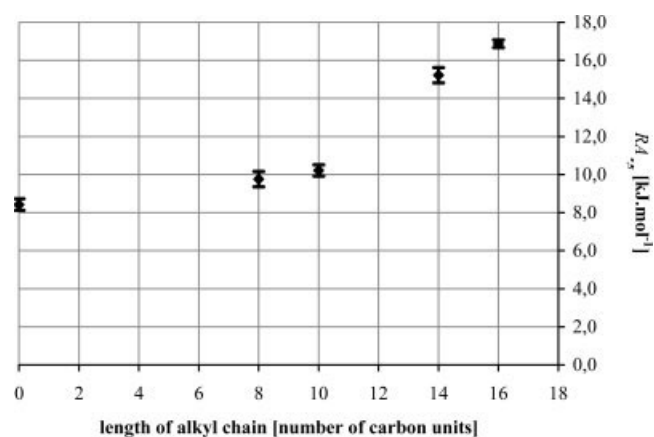


Figure 7. Dependence of voluminous expansion work of swelling $RA_{\delta,s}$ on alkyl chains length of Na-HA derivatives in water.

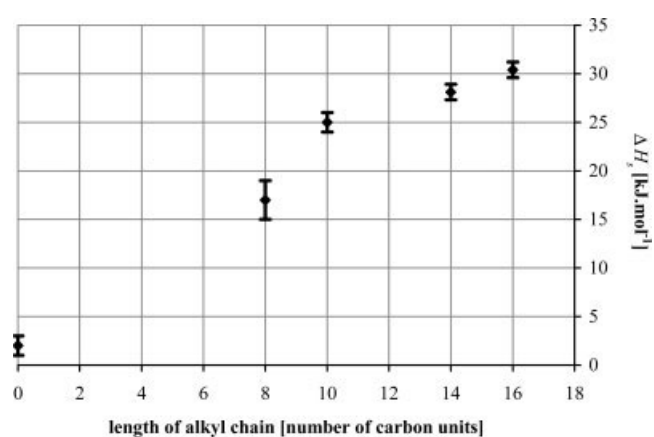


Figure 8. Dependence of activation enthalpy of swelling ΔH_s on alkyl chain length of Na-HA derivatives films in water.

influenced by the alkyl chain length. Experimental results of the apparent diffusion coefficients of water into the swelling hyaluronan showed rigorous increase of hydrophobic character of Na-HA derivatives. On the other hand, voluminous expansion work of swelling $RA_{\delta,s}$ increases with longer alkyl chains. Higher values of ΔH_d than ΔH_s can support the hypothesis, that Na-HA and its derivatives have a better swelling capacity, which decreases with increasing alkyl chain length than dissolution capacity. Relatively lower values of $RA_{\delta,s}$ can give further support to the latter hypothesis.

For further experiments, the authors are conscious of the necessity to evaluate the diffusion coefficients in dependence on the concentration profile obtained from interferograms²¹ and presently software system for plotting of the concentration profile is being developed. Nevertheless, the presented diffusion data of water into the hyaluronan and its derivatives films positively prove the hydrophobic character of alkyl derivatives of hyaluronan.

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