The behaviour of hyaluronan solutions in the presence of Hofmeister ions: A light scattering, viscometry and surface tension study

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ABSTRACT

Dynamic light scattering (DLS), viscosity and surface tension (SFT) measurements were used to characterize influence of salts containing ions of Hofmeister series (Na_2SO_4, $(NH_4)_2SO_4$, NaSCN, NH_4SCN and NaCl) on the behaviour of hyaluronan in diluted solutions at a temperature range of 15–45 °C. The results of the study showed that chaotropic and kosmotropic ions notably influenced the folding and unfolding of hyaluronan coils due to interactions between a respective ion and hydrophilic or hydrophobic patches present in the backbone of the polymer chains. This was mainly proved by viscosity and light scattering measurements. The temperature dependence of the hydrodynamic diameter of the hyaluronan coil determined by DLS demonstrated that combinations of chaotropic and kosmotropic ions in one salt (NaCl, NaSCN and $(HN_4)_2SO_4$) can stabilize the size of the coil in a wide range of temperatures. Tensiometry measurements indicated that certain types of ions present in the solution caused an unfolding of the hyaluronan coils, leading to a decrease of SFT.

1. Introduction

The interactions of ions with polymers in diluted solutions are challenging phenomena in colloid and interface sciences. The first systematic study dealing with the interactions of ions with rigid polymers, namely proteins, was published by Franz Hofmeister in 1888 (Baldwin, 1996; Hofmeister, 1888; Kunz, Henle, & Ninham, 2004). His pioneering research resulted in an arrangement of ions in a series with specific properties in terms of protein precipitation, and in the classification of ions into salting-in (chaotropic) and salting-out (kosmotropic) systems. However, theories related to principles and explanations of the described phenomena are still discussed, and interpretations of data on interactions between the ions and macromolecules, which are not rigid but exist as a statistic coil, are still not fully explained and are considered even more complicated than those on rigid proteins (Zhang & Cremer, 2006). Recently, the research groups of Cremer and Jungwirth published feature article (Okur et al., 2017) about ion-specific effects of Hofmeister ions on proteins and their biological function. The main message of the work (Okur et al., 2017) consists in identification of dominant role of strong/weak ion hydration, and binding affinity of the ion to charged side-chain group or to hydrophobic backbone of polymer chain. Okur et al. show experimental data and molecular dynamics simulations supporting their hypotheses. Moreover, the specific behaviour of ions in water in the absence of polymers, such as hydration, has been extensively studied both experimentally and theoretically (Barthel & Krestov, 1991; Bernal & Fowler, 1933; Franck, 1960; Heinzinger & Vogel, 1974; Hribar, Southall, Vlachy, & Dill, 2002; Hummer, Pratt, & García, 1998; Marx, Sprik, & Parrinello, 1997; Meyer & Pontikis, 1991; Payne, Teter, Allan, Arias, & Joannopoulos, 1992; Samoilov, 1957, 1972; Vlachy et al., 2009), and these interactions must also be taken into consideration under theoretical explanations of interactions of macromolecules with ions of the Hofmeister series.

The order of ions in the original Hofmeister series and their related properties are illustrated in Fig. 1. The left side of the series is comprised of kosmotropes, while chaotropes are on the right side. The terms "kosmotropic" and "chaotropic" ion/behaviour are related to the capability of a particular ion to "make" or "break" a water structure (Kropman & Bakker, 2003, 2004; Omta, Kropman, Woutersen, & Bakker, 2003a, 2003b). It is also generally accepted that these effects are more prominent for anions than for cations. However, with regard to a deeper understanding of the influence of ions on the behaviour of polymers, with the exception of proteins, the published data is rather scarce, concentrating prevailingly on thermoresponsive polymers
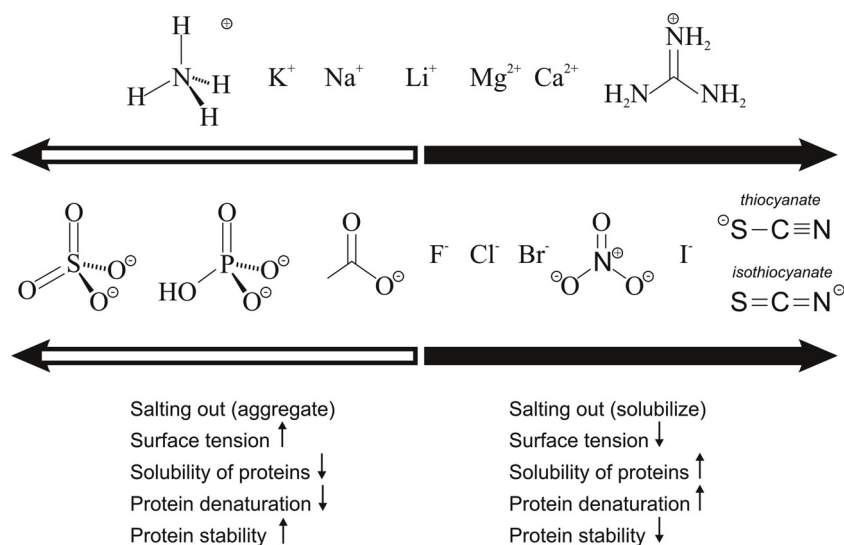
Fig. 1. Typical ordering of the Hofmeister ion series (Zhang & Cremer, 2006).

(Deyerle & Zhang, 2011; Rembert, Okur, Hilty, & Cremer, 2015; Thormann, 2012). Zhang et al., for example, published a theory and experimental data on the effect of specific Hofmeister ions on the solubility of (poly(N-isopropylacrylamide) (Zhang, Furyk, Bergbreiter, & Cremer, 2005). The authors concluded that the structure of bulk water is not significantly influenced by the nature of the salt present in the solution outside the first polymer hydration shell, and the influence of Hofmeister salts on the solubility of the polymer can be explained by interactions of the ions with the macromolecules and this first hydration shell. Strongly hydrated macromolecules do not facilely shed their innermost hydration shell and, hence, have the weakest binding constants for specific chemical groups of salts (Song, Ryoo, & Jhon, 1991; Von Hippel, Peticolas, Schack, & Karlson, 1973). Recently, a work dealing with the Hofmeister effect of NaCl and NaSCN on aqueous solutions of poly(propylene oxide) was published, which investigates the influence of molecular weights and concentrations of dissolved polymers on inter-chain interactions after the addition of salt (Moghaddam & Thormann, 2016). The experiments have led to the conclusion that increased molecular weight weakened the effect of both salts, which was ascribed to the scaling law between the molecular weight and the accessible surface area of the polymer. With respect to the influence of salts on polymers differing in concentrations, the effect of NaCl decreased while it enlarged for the NaSCN when the polymer concentration increased.

Hyaluronan or sodium hyaluronate (NaHy) is one of the most interesting biomacromolecules. This linear polysaccharide, composed of regularly alternating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamin, is almost ubiquitous in biological tissues (Balazs & Gibbs, 1970; Garg & Hales, 2004; Laurent & Fraser, 1992). Under physiological conditions, NaHy is assumed to exist in the form of a random coil possessing considerable stiffness, which was originally ascribed to a short persistence length ranging from 4 to 15 nm (Takahashi et al., 2003). The conformation of the macromolecule, however, can be affected by different variables including ionic strength, specific ion interactions, excluded volume effects or pH (Cowman & Matsuoka, 2005). Later on, hydrogen bonding between adjacent saccharide molecules in combination with the effect of electrostatic repulsion between carboxyl groups present alongside the polymer chain were determined to contribute to the stiffness of the chain (Morris, Rees, & Welsh, 1980; Wik & Comper, 1982). Thanks to this structure, NaHy molecules can form an ordered secondary structure, containing hydrophobic domains (patches) present in the backbone, and hydrophilic groups located in the side branches of the NaHy molecule (Scott, 1992). For the hydrophobic part of the molecule, hydrophobic

attractions must be considered, while interactions of the hydrophilic parts of the chain are influenced by hydration forces (Faraudo & Bresme, 2005). A comprehensive study summarising the behaviour of NaHy in diluted polymer solutions was published by Cowman and Matsuoka (Cowman & Matsuoka, 2005). Here, however, the behaviour of NaHy in a saline solution was mainly discussed, as this solvent, resembling *in vivo* physiological conditions, is mostly used for NaHy dissolution. Interactions of NaHy with other types of ions have been investigated only rarely, and a publication by Mracek et al. (2008) is an example. The influence of Hofmeister ions on polysaccharides, including NaHy, was also investigated by Tatini et al. (2017), who employed rheology and differential scanning calorimetry (DSC) for this purpose. The authors, however, used a more concentrated polymer solution (1%, w/w), which can hardly be compared with diluted systems. The DSC measurements were also used for analysing of structure, hydration and thermodynamic behaviour of NaHy with different levels of adsorbed water in solid or soft matter state of polymer (Albèr, Engblom, Falkman, & Kocherbitov, 2015; Průšová, Šmejkalová, Chytil, Velebný, & Kučerík, 2010).

The goal of this study was to contribute to understanding of interactions between Hofmeister ions and NaHy in diluted solutions. The conformation of NaHy, a statistic polymer coil, is strongly influenced by "structure making" and "structure breaking" properties of the ions, as well as by temperature. As water competes for hydrogen bonding present in a NaHy molecule, it will impact on the flexibility of the polymer coil, and hence on its hydrodynamic volume. This is reflected by changes of diameter of the coil, which can be determined by viscosity and light scattering measurements. The size and conformation of the polymer coil then impact on surface tension, which can also indirectly describe ion-coil interactions. In this study, the NaHy dissolved in aqueous solutions of salts containing selected ions of Hofmeister series will be studied using the three above mentioned techniques. Moreover, dependence of NaHy coil size on temperature will be also investigated in presence of these salts in order to elucidate possible conformation changes of the coil. The study can increase knowledge on behaviour of hyaluronan in ionic environment, which is prerequisite for application of this polymer in biological and technical fields.

2. Materials and methods

2.1. Materials

Sodium hyaluronate (NaHy), Mw = $1.8–2.1 \times 10^6$ g mol^{-1}, was the kind gift of Contipro Ltd. (Czech Republic). Sodium sulfate (Na$_2$SO$_4$),

ammonium sulfate ((NH$_4$)$_2$SO$_4$), sodium thiocyanate (NaSCN), ammonium thiocyanate (NH$_4$SCN) and sodium chloride (NaCl) were purchased from Sigma-Aldrich (Czech Republic). The purity of all salts used was higher than 97.5%, and they were used as delivered.

2.2. Sample preparation

Aqueous solutions of Na$_2$SO$_4$, (NH$_4$)$_2$SO$_4$, NaSCN, NH$_4$SCN and NaCl containing ions with specific positions in the Hofmeister series were prepared in concentrations corresponding to the ionic strength of 0.1 mol l^{-1}. The ionic strength I of a solution was calculated according to the equation $I = 1/2 \sum c_i z_i^2$, where c_i is the molar concentration of the ion i (mol l^{-1}), z_i is the charge number of the ion, and the sum is taken over all ions in the solution. Stock NaHy solutions with a concentration of 0.1% (w/w) were prepared by dissolving the polymer in the respective salt solution at 50 °C for 24 h under continuous stirring. Correspondingly, an NaHy solution in demineralised water was prepared.

2.3. Dynamic light scattering

Hydrodynamic diameters of NaHy coils were determined by dynamic light scattering (DLS) on a Zetasizer Nano ZS90 instrument (Malvern Instruments, Malvern, UK) and expressed as intensity-weighted z-average diameters (nm). Analyses were carried out on samples diluted in solutions of respective salts at a scattering angle of 173°. Prior to measurements, all samples were filtered through a 0.45 μm syringe filter (Millipore, UK). After filtering, the solution was left at rest for about 30 min to relax the polymer coil. Measurement of each of the samples was conducted using two procedures. At first procedure, z-average diameter was simply determined at 25 °C. In the second procedure, the dependence of z-average diameter on temperature was measured as follows. The filtered polymer solution, in properly sealed cuvette, was inserted into the instrument and cooled down to 5 °C. The instrument was programmed to automatically increase temperature from 5 to 65 °C, and z-average diameter was recorded with a step of $\Delta T = 1$ °C (temperature trend). At each temperature, the solution was equilibrated for 8 min. Three readings of the z-average diameter were performed and averaged to reach a final value. On the temperature trend, a conformation change of the NaHy coil from ordered to disordered state, visible as a "break point" with an abrupt change of z-average diameter was determined as the temperature point where the relative standard deviation (RSD) of the z-average diameter was higher than 10%.

2.4. Viscosity

Viscosity measurements were performed using an Ubbelohde capillary viscometer. The NaHy solution with the highest used concentration (1.0 × 10^{-3} g ml^{-1}) was gradually diluted with an aqueous solution of respective salt to obtain solutions with concentrations of 7.5 × 10^{-4}; 6.0 × 10^{-4}; 5.0 × 10^{-4} and 4.3 × 10^{-4} g ml^{-1}. Values of the limiting viscosity number (LVN) [η] and Huggins parameter k_H were determined from the flow times measured with these polymer solutions, and the linear least square regression of the sp/c vs. c dependence was used for the [η] and k_H calculations (c is polymer concentration [g ml^{-1}], η_{sp} is specific viscosity). The measurements were performed with NaHy dissolved at all studied salt solutions at temperatures of 15, 25, 30, 35 and 45 °C.

2.5. Surface tension

Measurements of surface tension were conducted using a programmable tensiometer (Kruss GmbH, Germany, Model: K20 EasyDyne) by the Wilhelmy plate method. The platinum plate was thoroughly cleaned with ethanol and flame-dried before each measurement. The surface tension of NaHy dissolved in salt solutions and water was measured at temperatures of 15, 20, 25, 30, 35, 40 and 45 °C. Readings were recorded each 15 s during the time interval of 300 s at each of the temperatures. At each time point, three readings were taken with a standard deviations not exceeding ± 0.1 mN m^{-1}. As a reference, the surface tension of deionized water at 25 °C was employed.

3. Results and discussion

3.1. Rationale for choice of salts

This work focuses on an investigation of the behaviour of NaHy in the presence of ions/salts of the Hofmeister series, namely Na$_2$SO$_4$, (NH$_4$)$_2$SO$_4$, NaSCN, NH$_4$SCN and NaCl. In this context, two important facts are worth mentioning: (1) there is not a single and unique series of Hofmeister ions. Some of the ions can change their position, and even a reversed or a bell-shaped series have been described (Omta et al., 2003b); (2) Hofmeister proposed his series only for salts and not for individual ions; it follows that each of the salts is a combination of cation and anion positioned at a specific place of the series. For this work, strongly hydrated kosmotropic (SO$_4^{2-}$, K$_A$) and weakly hydrated chaotropic (SCN$^-$, CH$_A$) anions were selected together with Cl$^-$ being placed roughly in the middle of the series, which is however still classified as a chaotropic ion. Correspondingly, a soft weakly hydrated cation (NH$_4^+$, CH$_C$) was used together with Na$^+$ (K$_C$), which forms a borderline between chaotropic and kosmotropic cations but is still included among kosmotropes. Combinations of these ions resulted in characteristic properties of selected salts consisting of the following: two chaotropic (NH$_4$SCN, CH$_C$–CH$_A$) and two kosmotropic (Na$_2$SO$_4$, K$_C$–K$_A$) ions, a combination of a chaotropic cation and kosmotropic anion ((NH$_4$)$_2$SO$_4$, CH$_C$–K$_A$), and a kosmotropic anion and chaotropic cation (NaSCN, NaCl, K$_C$–CH$_A$).

3.2. Dynamic light scattering

Size measurements showed different behaviour of NaHy dissolved in water and NaHy dissolved in all used salt solutions. In solely aqueous solutions, the NaHy coils were (to high extent) influenced by intramolecular and intermolecular electrostatic repulsions. As a consequence, the coils were big and volumes occupied by polymer chains were large. Most of the volume was, however, water not bound by the polymer (Cowman, Schmidt, Raghavan, & Stecco, 2015). In all salts solutions the coils were notably smaller, and their sizes depended on the type of ions/salts. The largest size 91 ± 5 nm was recorded for NaHy dissolved in NH$_4$SCN (CH$_C$–CH$_A$) followed by a second salt containing NH$_4^+$, namely (NH$_4$)$_2$SO$_4$ with a z-average diameter of 78 ± 2 nm. Interestingly, coil dimensions in all salt solutions with kosmotropic Na$^+$ were smaller. However, NaHy in the Na$_2$SO$_4$ solution containing both a kosmotropic cation and anion (K$_C$–K$_A$) behaved in a slightly different manner than NaHy in K$_C$–CH$_A$ salts, and its diameter was larger (68 ± 3 nm) than diameter of NaSCN (62 ± 1 nm) and NaCl (62 ± 1 nm).

The size of the NaHy coil was also determined in dependence on temperature (Fig. 2). When heated from 5 to 65 °C, a solution of NaHy in water performed again differently in comparison with NaHy in salt solutions. This is in accord with previously reported results showing that the hydrodynamic diameter of NaHy dissolved in water was rather unstable (Grundelova, Mracek, Kasparkova, Minarik, & Smolka, 2013). From the statistical point of view, the values of z-average diameters of the NaHy coil measured at each temperature in water were not comparable, and significant variations were observed. This is depicted in Fig. 2a, showing random changes of the z-average diameter of NaHy in water during heating and large standard deviations observed for measurements performed at each temperature. Similar variations have previously been reported by Schurz, Hemmetsberger, Sasshofer,
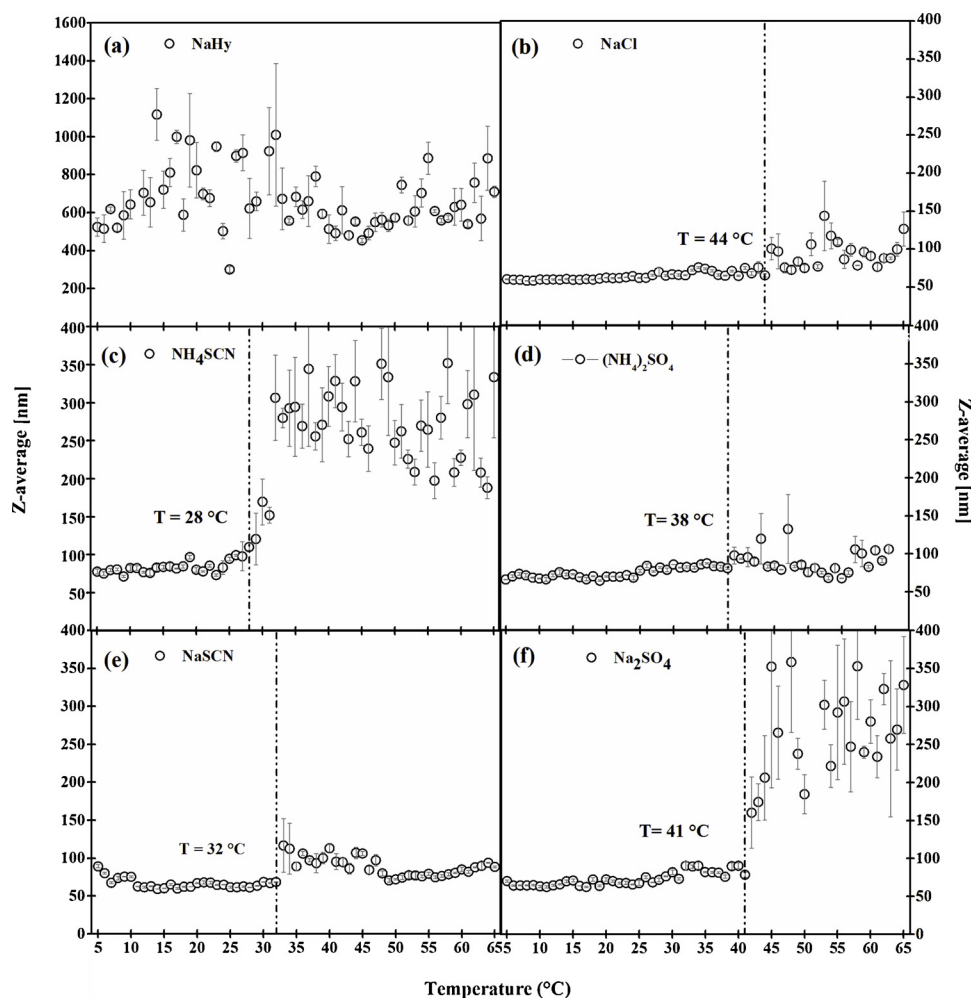
Fig. 2. Temperature dependence of coil size of NaHy dissolved in (a) water and aqueous solutions of salts containing ions of the Hofmeister series (b) NaCl (K_C–CH_A); (c) NH_4SCN (CH_C–CH_A); (d) $(NH_4)_2SO_4$ (CH_C–K_A); (e) NaSCN (K_C–CH_A); (f) Na_2SO_4 (K_C–K_A).

Tomiska, and Tritthart (1967) who found out that NaHy dissolved in water can exist in two states differing in the intrinsic-viscosity/molecular weight relation, which correspond to loose coils and dense particles. Also Ribitsch, Schurz, and Ribitsch (1980) reported on complex behaviour of NaHy dissolved in water without salts present. They concluded that NaHy forms different solution structures in different solvents, and the solutions were reported to undergo significant changes in the course of time. Existence of stable solutions occurred only under very special conditions; in their work high ionic strength was mentioned as a prerequisite for stable behaviour of the NaHy coil. The performance of NaHy observed in our work complies with these findings. On the contrary, NaHy in salt solutions showed more uniform performance with a certain "break point" at the z-average vs. temperature dependence, at which the polymer coils changed their behaviour. With respect to the size of the NaHy coil, these systems were rather stable before the "break point", which is also evidenced by low standard deviations of measured values before the break, whereas they appeared to be non-equilibrium systems beyond this point. It was determined that the temperature of the "break point" in the presence of the studied salts decreased in the following order: NaCl (K_C–CH_A 44 °C) > Na_2SO_4 (K_C–K_A 41 °C) > $(NH_4)_2SO_4$ (CH_C–K_A 38 °C) > NaSCN (K_C–CH_A 32 °C) > NH_4SCN (CH_C–CH_A 28 °C). Beyond the "break point", the biggest coil diameters were recorded for NaHy dissolved in salts combining CH_C–CH_A (NH_4SCN) and K_C–K_A (Na_2SO_4) (Fig. 2c and f). On the contrary, Fig. 2b, d and e demonstrate that the salts containing combinations of K_C–CH_A or CH_C–K_A play a significant

role in stabilizing the NaHy coils. This can be concluded from minor changes in their sizes with temperature observed beyond the "break point". This observation can lead to the hypothesis that combinations of chaotropic and kosmotropic ions in one salt might actually control conditions for the folding/unfolding of the NaHy coils due to a balance between the influence of kosmotropic and chaotropic ions (Marcus, 2009).

As already mentioned, the highest break point temperature (44 °C) was recorded for NaHy dissolved in NaCl. Moreover, in this case NaHy behaved consistently throughout the entire studied temperature range. It may be therefore speculated that this can contribute to the stable behaviour of NaHy in physiological conditions of the body. In light of these observations, it seems that NaCl creates optimum conditions for the stability of the polymer coil, and the Cl^- anion plays only a minor role as a chaotropic anion. Generally, the effect of anions with respect to hydration is more significant than that of cations because anions interact more strongly with water than cations of the same charge density (Collins, 1997) (Table 1).

3.3. Viscosity

A comparison of the LVN values of NaHy dissolved in water and salt solutions showed a significant difference. The viscosity determined in water was notably higher (5940 ml g^{-1}) than viscosities in all salt solutions, which were lower than 2800 ml g^{-1}. The higher viscosity of NaHy in water arose from the different conformation of polymer chain

Table 1
Average size of NaHy coil ± standard deviation determined in water and in aqueous solutions of Hofmeister ions/salts. Break at *z*-average diameter vs. temperature dependence (temperature trend) corresponds to temperature where the relative standard deviation (%) of *z*-average values is higher than 10%.

Salt added to water	Types of ions present	*z*-Average diameter at 25 °C [nm]	Break at "temperature trend" [°C]
None	None	286 ± 7	Absent
NH₄SCN	CH$_C$–CH$_A$	91 ± 5	28
(NH₄)₂SO₄	CH$_C$–K$_A$	78 ± 2	38
NaSCN	K$_C$–CH$_A$	62 ± 1	32
NaCl	K$_C$–K$_A$	62 ± 1	44
Na₂SO₄	K$_C$–K$_A$	68 ± 3	41

in this solvent. While in water NaHy chains are stretched, they shrink in the presence of salts, and their hydrodynamic volume decreases, resulting a drop in the LVN. Better packing of the NaHy in salt solutions is caused by the shielding of the electrostatic repulsions between similar charges located along the polymer chain. At a sufficiently high ionic strength, the charges due to the carboxylate groups on the NaHy chain are completely screened, thus diminishing the repulsion between them, which hinders expansion of the coil. In water, however, the repulsions increase the hydrodynamic volume of the coil, and viscosity increases. The LVNs of NaHy dissolved in salt solutions at temperatures ranging from 15 to 45 °C are given in Fig. 3 which illustrates bigger expansion of NaHy coil in sulfates (Na₂SO₄, (NH₄)₂SO₄) than in thiocyanates (NaSCN, NH₄SCN) and NaCl. Behaviour of polymer is consistent at almost all studied temperatures and can be correlated with the compositions of the used salts. At temperatures of 15, 25, 30, 40 °C, the LVN decrease in the following order, Na₂SO₄ (K$_C$–K$_A$) > (NH₄)₂SO₄ (CH$_C$–K$_A$) > NaSCN = NaCl (both K$_C$–CH$_A$) > NH₄SCN (CH$_C$–CH$_A$), which conforms to the position of individual ions in the Hofmeister series. Viscosity measurements also show that the impact of anions on the behaviour of NaHy is more notable in comparison with cations. An exception was observed for LVNs measured at 35 °C, where the lowest viscosity exhibited NaHy dissolved in NaCl, and viscosities of (NH₄)₂SO₄, NaSCN and NH₄SCN were of similar values. In this context it can be mentioned that sulfates belong to kosmotropic ions capable of subtracting water from the hydration layer of polymers. Therefore, in the case of NaHy with negatively charged carboxylic groups, the presence of kosmotropes weakens the polymer network, which decreases

the strength of the polymer–polymer interactions and leads to coil expansion. On the other hand, chaotropic thiocyanates, which can disrupt the structure of bulk water, will contribute to coil shrinkage, resulting in a decrease of viscosity. In addition to the influence of anions, the impact of chaotropic or kosmotropic cations in the salt will contribute to the above described behaviour of the NaHy. In the feature article published by Okur et al. (2017) the authors thoroughly discussed the hydration properties of individual ions of Hofmeister series and their interactions with polymers, mainly proteins. However, behaviour of aqueous solutions of NaHy and proteins in presence of salts is difficult to compare. System "sodium hyaluronate-salt/ions-water" is rather complex, and wide range of different interactions, which can hardly be separated from each other occurs.

The size of a polymer coil (LVN) is governed by stiffness of the polymer chain. In the case of NaHy, this mainly depends on the rotation of the linkages between sugar monomers, which is easier with increasing temperature, and thus at higher temperatures the polymer chains show higher flexibility. This reduces molecular volume, and consequently lowers the viscosity (Cowman et al., 2015). In their works Cleland (Cleland, 1979) and Fouissac, Milas, and Rinaudo (Hoefting, Cowman, Matsuoka, & Balazs, 2002) reported that the LVN of high molecular weight hyaluronic acid decreased by about 25% as the temperature was increased from 25 to 65 °C, which corresponds to a decrease of about 0.63% per 1 °C. The NaHy studied in this work, however, showed a milder decrease in LVN across the temperature range (15–45 °C), and viscosity decreased by 0.45% per 1 °C. This difference might be simply explained by differences in molecular weight of the polymers used in the test.

In addition to the LVN, viscosity data afford also Huggins coefficient (k_H). The k_H describes the polymer–solvent interactions and values of around 0.3 and 0.8 to 1.0 are typical for polymers dissolved in good and bad solvents, respectively. Values of k_H decrease with increasing expansion of the polymer coil α_η, which then depends on the molecular weight of a polymer and the strength of the polymer–solvent interaction (Bohdanecký & Kovář, 1982). Cowman and Matsuoka (Cowman & Matsuoka, 2005) summarised and reported k_H values for NaHy dissolved in NaCl determined at 25 °C. Based on previously published scientific works, they found k_H ranging from 0.33 to 0.57. Taking into consideration the theoretical value of $k_H = 0.4$, the k_H values determined in this work were slightly lower (0.25–0.33), and no systematic dependences either on temperature or salt type were observed. These data however indicate that all the used salt solutions are good solvents for NaHy samples.
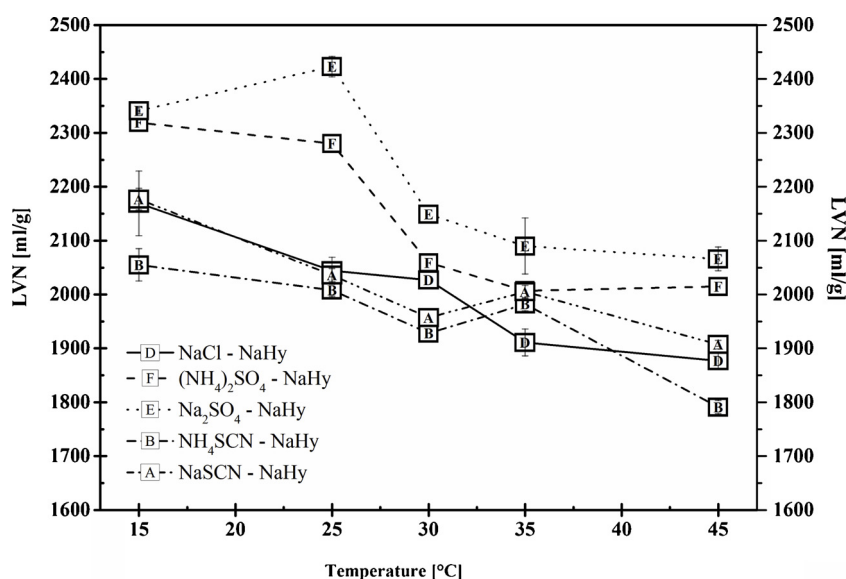

Fig. 3. Dependence of LVN on temperature determined for NaHy dissolved in salt solutions (some values of A and D are overlapping).
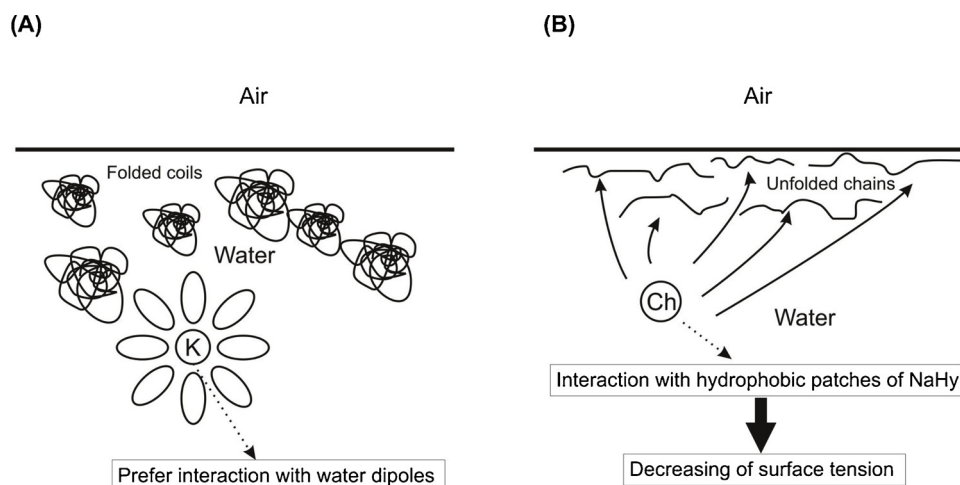
(A)   **(B)**

Fig. 4. Organisation of macromolecules on the air/water interface (the orientation of water dipoles depends on the kosmotropic cation/anion).

3.4. Surface tension (SFT)

Prior to discussing results from SFT measurements, it should be emphasized that this technique reflects the organisation of polymer chains on the air/water interface, not in a bulk solution as do the previous techniques. As such, a comparison of results from SFT and DLS/viscometry can be challenging. Nevertheless, chaotropic ions (supporting hydrophobic interaction within NaHy chains) or kosmotropic ions (supporting hydrophilic interaction between NaHy chains and water) influence the folding/unfolding of polymer coils and self-organisation of the NaHy at the air-water interface (Fig. 4) (Grundelova et al., 2013; Salvi, De Los Rios, & Vendruscolo, 2005). Therefore, the ion-coil interactions and the size of NaHy hydrodynamic diameter can indirectly influence SFT.

Fig. 5a compares values of SFT determined for water and for NaHy dissolved in water and in salt solutions, all measured at temperatures ranging from 15 to 45 °C. Fig. 5b then adds the measurements of SFT for the above systems after 300 s from the measurement start. In general, all samples, both immediately after preparation and after 300 s, behaved as expected, and SFT of all samples gradually decreased with increasing temperature. At the start of the analysis, the highest SFTs showed NaHy dissolved in water, and the lowest SFTs were measured in water alone. The presence of salts in water used for the dissolution of NaHy then decreased the SFT relatively to NaHy dissolved in water without salts. However, the values never dropped below the SFT of water alone.

Interesting information on the behaviour of NaHy in salt solutions can be gathered from the dependence of SFT on temperature. Both at measurement start and after 300 s, Fig. 5 indicates that the studied temperature region can be roughly divided into two intervals. The first region spans from 0 to 30 °C, and the second one covers the temperatures 35, 40 and 45 °C. At a temperature of 35 °C, NaHy starts to perform in a different way, and it appears that temperatures above 35 °C affect coil behaviour more significantly in comparison with lower temperatures. Of course, in both these regions the SFT of NaHy varies also with the type of salt used. In contrast with DLS and viscosity measurements, no uniform and systematic pattern in the behaviour of NaHy dissolved in the studied salt solutions was observed.

More detailed inspection of the results presented in Fig. 5a shows that at the start of the measurements and at temperature range 15–30 °C, the SFT of NaHy decreased in the following order: Na_2SO_4 $(K_C–K_A)$ > NaCl $(K_C–CH_A)$ > NH_4SCN $(CH_C–CH_A)$ > NaSCN $(K_C–CH_A)$ > $(NH_4)_2SO_4$ $(CH_C–K_A)$. It is also seen that the differences in the SFT measured for NaHy in NH_4SCN, NaSCN and $(NH_4)_2SO_4$ were only minor. At temperatures above 30 °C, the SFT of NaHy in salt solutions continued to gradually decrease with increasing temperature,

however the ways of the decrease in the studied salts differed.

After 300 s (Fig. 5b) the situation was, however, different. While SFT of water remained unaffected and was the same as at the measurement start (t = 0, Fig. 5a), the SFT values of NaHy solutions changed and these changes were influenced by the nature of the ions present in the solution. The SFTs of NaHy dissolved in water were no longer the highest of the measured values, as observed at t = 0. At 15 °C the SFTs of NaHy were rather similar in all salt solutions tested. When temperature increased to 20 °C, rather abrupt decrease of the SFT (from 73.8 to 71. 3 mN/m) for NaHy in NH_4SCN $(CH_K–CH_A)$ was measured. By contrast, the SFT of the polymer in NH_4SCN solution remained almost at the same value up to 35 °C, and then it decreased again. Behaviour of NaHy dissolved in the NaSCN $(K_C–CH_A)$ follows the pattern mentioned above, that is the different behaviour at the two mentioned temperature ranges. A mild decrease of the SFT was detected between 15 and 30 °C, after which the SFT dropped suddenly and showed the lowest value at 45 °C.

With respect to the observed behaviour of NaHy, and taking into account the structure of its molecule containing hydrophobic and hydrophilic parts, ions supporting hydrophobic interactions can be organised nearby these hydrophobic patches and can support the unfolding of NaHy chains, resulting in increase of the hydrodynamic diameter of the coil and decrease of the SFT.

4. Conclusion

The behaviour of NaHy in aqueous solutions containing Hofmeister ions is complex and depends on different variables. At present, experimental data dealing with this topic are scarce and insufficient for a deep understanding of this phenomenon. However, based on the results of this study, some general conclusions can be drawn. Viscosity measurements showed that the expansion of the NaHy coil is bigger in kosmotropic sulfates than in chaotropic isothiocyanates, which can be related to ability of these anions to act as "structure making" and "structure breaking factors. The shrinkage or expansion of the coil can be also attributed to interactions between the respective ion and hydrophilic or hydrophobic patches present in the backbone of the NaHy chain. Interesting information can be drawn from the DLS measurements as well. The main point here is notable difference in the "conformation stability" of the coils, in terms of their z-average diameters in dependence on temperature and type of ion present in the NaHy solutions. The results show that the combination of the kosmotropic cation and chaotropic anion (and *vice versa*) in one salt brings about the conformational stability of the NaHy chain within a wide range of temperatures. Finally, SFT measurements indicates that Hofmeister ions present in the NaHy solutions caused the unfolding of the NaHy coils,
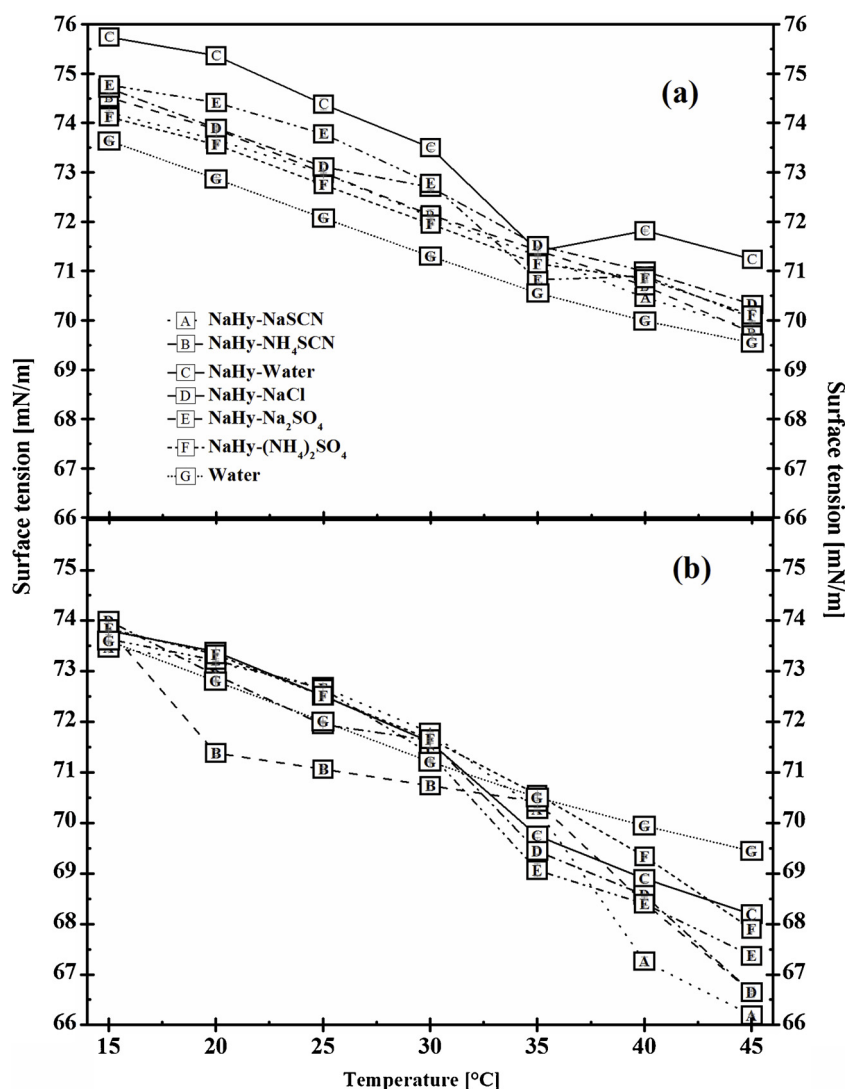
Fig. 5. Dependence of SFT of NaHy dissolved in salt solutions, NaHy dissolved in water and water alone (reference) on temperature (a) at measurement start ($t = 0$ s) and (b) after 300 s.

leading to a decrease of the surface tension.

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