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Influence of sodium hyaluronate on dehydration and water distribution in soft contact lenses

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The purpose of this investigation was to examine the influence of sodium hyaluronate (HA) solution on contact lens dehydration and the distribution of water in lens materials. These parameters were measured with gravimetry and differential scanning calorimetry. Five commercial soft contact lenses were used. They represented four FDA (Federal Drug Administration) groups: Air Optix Night & Day Aqua and Acuvue Oasys (I FDA group), Proclear 1-Day (II FDA group), PureVision (III FDA group) and 1-Day Acuvue Moist (IV FDA group). All materials were investigated with two preservative-free HA solutions 0.1% and 0.3%. HA solutions influenced the water content and the dehydration rate of some examined lenses. For three lenses (Oasys, Proclear, Moist) water content of HA lenses was greater than control. Significant slowdown of dehydration rate under HA during the first 20 min was observed only for Proclear. Phase I of dehydration increased significantly with HA solutions in case of Moist and Proclear. For Night & Day and Oasys phase I appeared under HA solution while it was not present for control lenses. Duration of the phase I was strongly correlated with water content of the lenses ($R^2 = 0.844$). The amount of freezable and non-freezable water depended strongly on characteristics of lens material and its interaction with HA molecules. Proclear seems to be the most prone to attach HA molecules which affect changes in dehydration characteristics and water behavior in the polymer. PureVision might be considered as the most resistant to HA in terms of dehydration dynamics and water distribution. All measured parameters seem to be dependent more on material properties than HA concentration.

Keywords: contact lens dehydration, sodium hyaluronate, gravimetry, differential scanning calorimetry.

1. Introduction

Dry eye symptoms are more prevalent in contact lens users than non-contact lens users [1] and they affect up to 50% of wearers who suffer from discomfort, usually reported as dryness [2–4]. The symptoms can be severe enough to reduce wearing time or cease lens wear [1].

The contact lens separates tear film into two layers, breaking its integrity and increasing evaporation [5]. Excessive evaporation of pre-lens tear film results in exposed lens surface susceptible to deposits and dehydration which is thought to play a role in comfortable lens wear.

Dehydration defined as a loss of water begins right after placing the contact lens on the eye and continues during the day, depends on the contact lens design and material properties as well as environment characteristics [6]. Lens dehydration may lead to the change of some important attributes like curve radius, thickness, permeability (in case of conventional lenses), changes in surface wettability [7, 8]. In consequence, loss of water can influence fitting characteristics [9] and might be also associated with corneal dessication and corneal surface damage [10–12].

In order to reduce dryness and irritation, soft contact lens users commonly use rewetting eye drops and many practitioners recommend them as a first choice treatment for dry eye symptoms [13]. It is estimated that about 20% of contact lens wearers use ocular lubricants to ease discomfort appearing usually at the end of the day [14]. Currently numerous rewetting eye drops exist on the market. Vast part of them contains sodium hyaluronate as a main lubricating and demulcent agent with concentration range from 0.1% to 0.4%.

Hyaluronan (HA) is a naturally occurring linear biopolysaccharide with exceptional hygroscopic, lubricating and viscoelastic properties which is present in human tear fluid [15]. In the eye HA protects corneal epithelium, accelerates cell migration and wound healing [16]. Its unique ability to attract and retain water makes it a good ingredient of ophthalmic products such as rewetting eye drops giving relief in ocular symptoms and dryness [15, 17]. Previous studies proved the protective and demulcent effect of HA on treatment of dry eye symptoms in non-contact lens users [18–20].

VAN BEEK *et al.* investigated HA incorporated into the contact lens materials which allowed to enhance wettability and decrease protein adsorption [21–23]. However, there is still little knowledge about interaction of HA solutions and soft contact lens materials and the way in which HA influences distribution of water in the polymer. As mentioned above, researchers have so far focused more on HA effect on the dry eye (non-contact lens wearers) or use of "lubricants" (saline drops) during contact lens wear [14, 24].

It seems essential to evaluate and understand potential behavior of different contact lens materials in the presence of ingredient that is used so often by contact lens wearers. Despite the diversity of available materials and widespread use of HA solutions, clinicians do not have indicators of the ability (or lack of thereof) of different lenses to interact with HA. Investigation of factors which may potentially influence the contact lens wear performance seems to be more significant in view of recent reports about numerous dropouts [25, 26]. It appears that HA may increase water content and slow down dehydration due to its ability to attract and retain water.

In the present work five different types of contact lens materials were investigated with HA solution in two concentrations. The purpose was to examine the influence of HA solution on contact lens dehydration and determine distribution of water (states of water) in polymer. These parameters were measured with gravimetry and differential scanning calorimetry (DSC).

2. Materials and methods

2.1. Materials

Five types of commercial soft contact lenses were used in this study: three silicone-hydrogel and two hydrogel. They represented four FDA (Federal Drug Administration) groups: Air Optix Night & Day Aqua and Acuvue Oasys (both I FDA group), Proclear 1-Day (II FDA group), PureVision (III FDA group) and 1-Day Acuvue Moist (IV FDA group). The choice of the two lenses from first group was motivated by different enhancements of the surface wettability. Details of the examined lenses are summarized in Table 1.

Table 1. Parameters of contact lens used in this work.

Contact lens brand	Material	Composition (main monomers) [6, 28]	WC [%]	FDA group	Surface treatment	*Dk*
Air Optix Night & Day Aqua	Lotrafilcon A	TRIS, PDMS, NVP, DMA	24	I	Plasma coating	140
Acuvue Oasys	Senofilcon A	HEMA, PDMS, DMA, PVP	38	I	No	103
Proclear 1-Day	Omafilcon A	HEMA, PC	60	II	No	32
PureVision	Balafilcon A	TRIS, NVP, TPVC, NCVE, PBVC	36	III	Plasma oxidation	99
1-Day Acuvue Moist	Etafilcon A	HEMA, MAA, TMPTMA, EGDMA	58	IV	No	28

WC – water content, FDA – Federal Drug Administration, DMA – N,N-dimethylacrylamide, EGDMA – ethylene glycol dimethacrylate, HEMA – 2-hydroxyethyl methacrylate, MAA – methacrylic acid, MPDMS – monofunctional polydimethylsiloxane, PVP – polyvinylpyrrolidone, PC – phosphorylcholine, PVA – polyvinyl alcohol, TEGDMA – tetraethyleneglycol dimethacrylate, TMPTMA – trimethylolpropane trimethacrylate.

All contact lenses were investigated with two preservative-free HA solutions at concentrations 0.1% and 0.3% due to the typical concentration of HA in ocular lubricants.

They were prepared with sodium hyaluronate molecular weight 50 kDa, proteins < 0.05% (Unispec Chemicals). It was expected that the low molecular weight HA would be faster released from lens material [21], however, the methods used in the present study did not require rinsing or flushing. Consequently HA molecules were not washed out from the pores of the lens.

2.2. Preparation of the lens

Before measurement all tested and control lenses were blotted with filter paper in order to remove the excess water. Subsequently the lens was placed on a plastic convex holder with curvature similar to the lens radius. It enabled to simulate the lens on the cornea with only anterior surface exposed to the air. Before DSC measurement 10–14 mg samples were cut and immediately hermetically sealed in aluminum pans to avoid dehydration.

The tested lenses (HA lenses) additionally were dipped for 3–5 seconds in one of HA solutions, again blotted and then measured. Three seconds dipping aimed to imitate typical application of eye drops and their contact with contact lens.

2.3. Measurements

Gravimetric measurements were conducted in Department of Medical Physics, Faculty of Physics, Adam Mickiewicz University in Poznań, Poland. A digital analytical balance was used (WPA 120/C/1, Rad Wag, Radom, Poland) with scale accuracy 1×10^{-4} g. Measurements were performed at room temperature 23 ± 1°C. The weight of each lens was registered every 60 s. Dehydration time for each type of lens was expected to be different and therefore the measurement was ended when the lens reached stable weight for at least 7 min (no change of lens weight observed). In each trial, samples of 5 to 6 lenses were examined and each sample was tested once.

In order to analyze gravimetry results, different parameters were derived.

Water content (WC) was determined using the following equation:

$$\mathrm{WC} = \frac{m_1 - m_2}{m_1} \times 100\% \tag{1}$$

where m_1 denotes weight of hydrated lens and m_2 – weight of dehydrated lens.

Dehydration rate (DR) represents the rate of weight loss for each lens per minute during dehydration process

$$\mathrm{DR} = \frac{m_t - m_{t-1}}{m_t} \times 100\% \tag{2}$$

where m_t denotes the sample weight at time t, and m_{t-1} denotes the sample weight at time $(t-1)$.

DR parameter was used to determine duration of phase I and II of dehydration. Phase I characterizes relatively high and stable DR, phase II begins when rapid decrease of DR parameter is observed [6].

Differential scanning calorimetry (DSC) was used in order to determine and monitor changes in water structure that may occur in presence of HA. The thermodynamic behavior of each type of water shows its dynamics in the polymer. TRANOUDIS and EFRON distinguished three classes of water: *bound water* creates direct hydrogen bonds with the polar groups of the polymer, *free water* does not interact with polymer and is able to participate in diffusion, *loosely-bound water* is interfacial and remains in liquid state slightly below 0°C [9]. The presence of rewetting substance may influence the proportion of individual type of water, its mobility in the polymer and in consequence determine physical properties of the lens material.

DSC measurements were conducted in Departmental Laboratory for Structural Research WLBS, Faculty of Physics, Adam Mickiewicz University in Poznań, Poland. DSC Q2000 (TA Instruments, New Castle, DE) heat flux type was used in this study (scale accuracy of heat flux 0.2 μW and scale accuracy of temperature ±0.05°C). The samples were scanned from +20°C (room temperature) to –40°C where phase transition of free and loosely bound water can be observed. This range of temperatures allows to compare the results with those from [9, 28]. Each sample underwent cycle as below.

Cooling to –40°C with a cooling rate 2.5°C/min, stabilization at temperature –40°C for 10 min, heating to +20°C with a heating rate 2.5°C/min, stabilization at temperature +20°C for 5 min. Measurement was repeated twice and the data was averaged. DSC method demonstrates good repeatability because of precision of the measurement and numerous pieces of the tested lens that can be sealed in the pan [9, 28].

The area under the peaks from melting endotherm was used to calculate the percentage of freezable water (free and loosely bound),

$$C = \frac{\Delta H_{\mathrm{tr}} k}{m \Delta H_f} \tag{3}$$

where C denotes concentration of water, ΔH_{tr} – heat of transition [mJ], m – sample weight [mg], k – cell calibration coefficient (dimensionless), ΔH_f – heat fusion of water (333.7 J/g).

The amount of bound water was obtained by subtraction of the freezable water content (WC) from the total water content calculated from gravimetry

$$\text{Bound WC [\%]} = \text{Total WC [\%]} - \text{Freezable WC [\%]} \tag{4}$$

3. Results

Data were tested for normality of distribution using Shapiro–Wilk tests ($p < 0.05$). One-way analysis of variance (ANOVA) was used to compare the WC and phase I duration of control and examined lenses. For all testing, the significance level $p < 0.05$ was considered significant. Analyses were performed using Origin software version 9.1 (Origin Lab Corporation, Northampton, MA).

Figure 1 represents WC of examined lenses. Lenses soaked in HA solutions increased significantly their water content comparing to control lens for hydrogel lenses: Proclear and Moist control *vs*. 0.1% HA ($p = 0.029$ and $p = 0.035$, respectively) and control *vs*. 0.3% HA ($p = 0.013$ and $p = 0.017$, respectively). Significant difference was observed also for Oasys although only with 0.3% HA ($p < 0.01$). According to

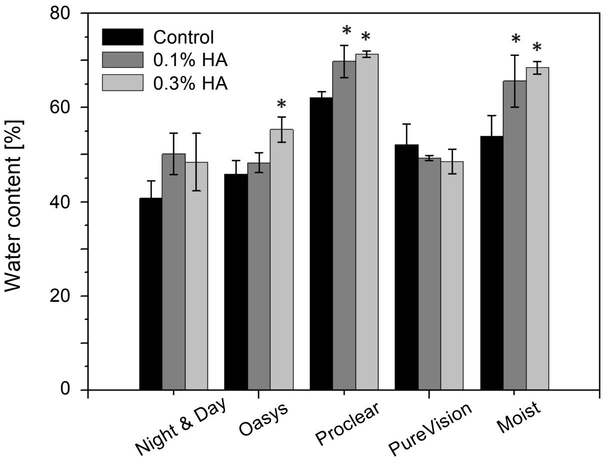

Fig. 1. Comparison of water content among three groups: control lenses without any solution (black bars), lenses soaked in 0.1% HA (grey bars) and to 0.3% HA (light-grey bars); data show the mean and ±SD (error bars); * – $p < 0.05$ ANOVA test for the difference between control group and HA solution.

Fig. 1, the greatest increase of water content with HA was observed for Moist (15.0% with 0.3% HA) and Oasys (11.6% with 0.3% HA). For Night & Day water content increased although there was no significant difference ($p = 0.111$ and $p = 0.207$). Surprisingly PureVision water content diminished with HA, even though this decrease was not significant ($p = 0.674$ and $p = 0.535$).

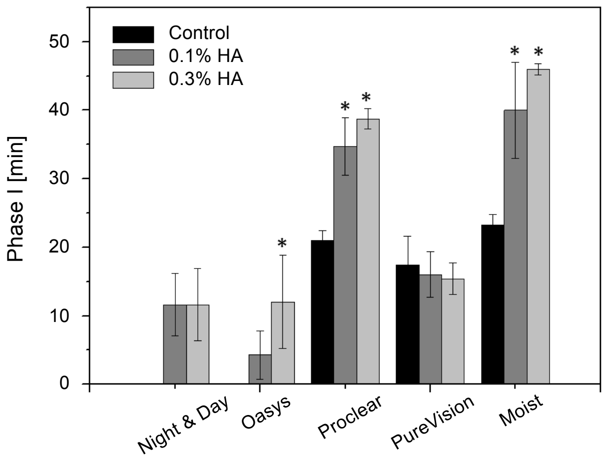

Fig. 2. Comparison of phase I of dehydration among three groups: control lenses without any solution (black bars), lenses soaked in 0.1% HA (grey bars) and to 0.3% HA (light-grey bars). Phase I was not observed in case of control Night & Day and Oasys lenses. * – $p < 0.05$ ANOVA test for the difference between control group and HA solution.

From the DR values the duration of phase I was determined. Duration of phase I for each lens is presented in Fig. 2. Significant increase in phase I duration with 0.1% HA and 0.3% HA was observed for Moist ($p < 0.01$ and $p = 0.017$, respectively), Proclear ($p < 0.01$ and $p < 0.01$, respectively) and Oasys but only for 0.3% HA ($p = 0.020$). In case of control lenses Night & Day and Oasys (both I FDA group) phase I was absent, while it appeared with both HA solutions. Duration of phase I is plotted in Fig. 3 against the equilibrium water content (EWC) displaying correlation (Pearson coefficient $R^2 = 0.844$, $p < 0.01$). The differences were not found in phase II for any of the examined lens ($p > 0.05$).

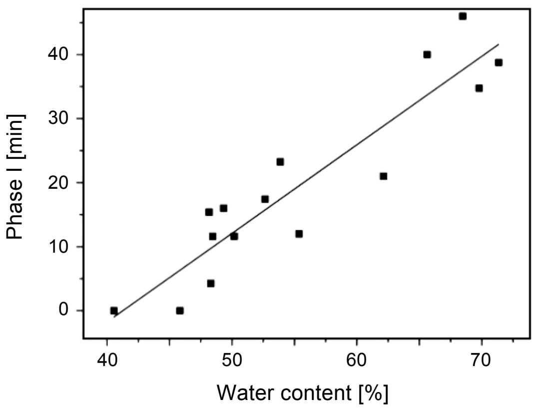

Fig. 3. Relationship of lens material water content with duration of phase I. Figure presents length of phase I for each lens type (Fig. 2) in function of EWC (Fig. 1). Fifteen points correspond to all types of the control, 0.1% HA and 0.3% HA lenses.

In order to determine short-term dehydration characteristics which might reflect potential behavior of the lens material *in vivo*, the first 20 min were divided into 5 min periods and mean DR value was calculated. Results presented in Fig. 4 indicate that only for Proclear dehydration rate slows down significantly in each period. Oasys HA demonstrated higher DR value to control in 15–20 min period. In case of other lenses no significant changes were observed.

Figure 5 shows exemplary DSC results for two contact lens materials (Proclear, Night & Day) to present two patterns of endothermic curves – with one and two peaks. On Oasys, Proclear, and Moist melting endotherm curves two peaks were observed. One sharp peak at about 0°C and another broad peak at about –5°C to –10°C which correspond to free and partially bound water, respectively. In case of Night & Day and PureVision only one peak was present at 0°C. The area of each peak was estimated from the difference between melting endotherm and a baseline drawn in the figure using the computer program that controls the DSC measurements. Using Eq. (3), the amount of water was calculated (Table 2).

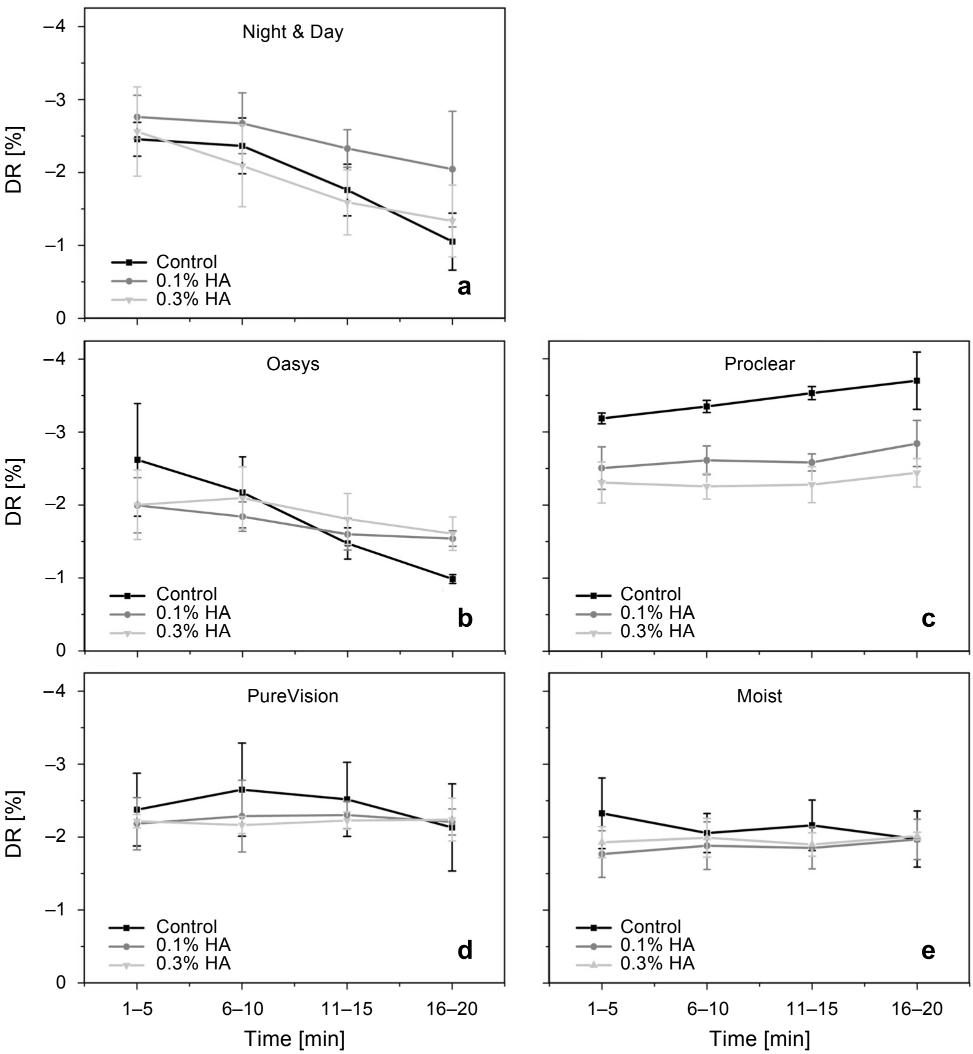

Fig. 4. Mean dehydration rate (DR) at 5 min intervals for the first 20 min: Night & Day (**a**), Oasys (**b**), Proclear (**c**), PureVision (**d**), and Moist (**e**). Vertical bars present standard deviation.

In case of four lenses (Night & Day, Oasys, Proclear, PureVision) the area of the peaks increased for HA samples, which reflects the increase of free and loosely bound (freezing) water content. However, the higher concentration of HA is not related to greater increase of free water amount. HA solution influenced also the increase of bound (non-freezing) water for Night & Day, Oasys, Proclear and Moist. PureVision exhibits different pattern – HA samples contained less non-freezing water than control. Consequently three types of relationship were observed:

– increase of free and bound water content in presence of HA solution (Night and Day, Oasys, Proclear),

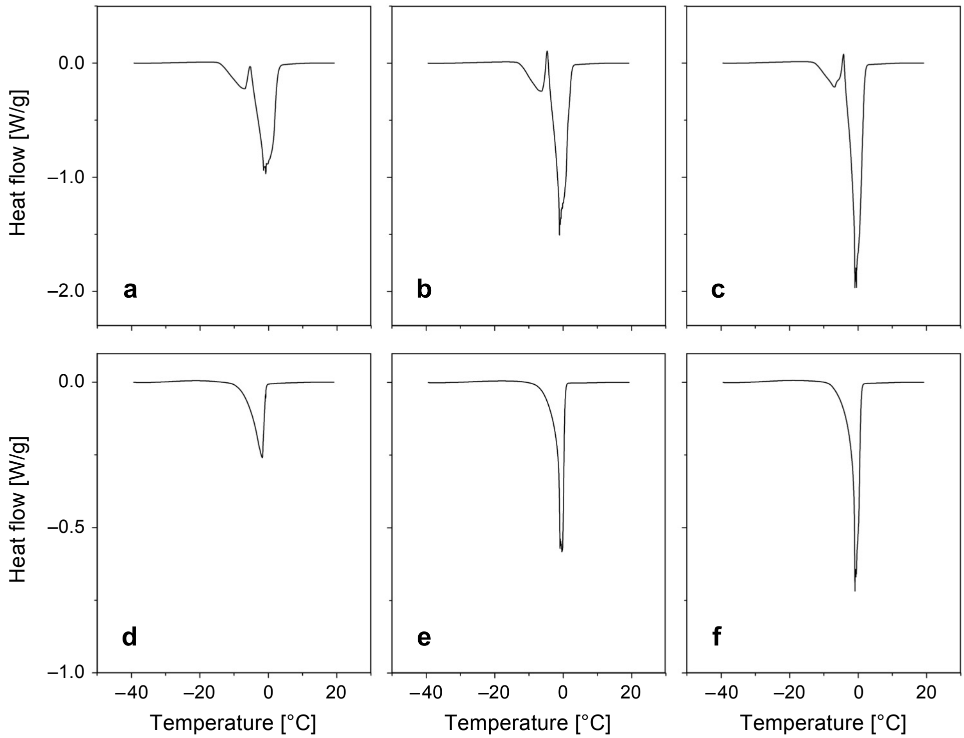

Fig. 5. Exemplary DSC melting endotherms for Proclear: control (**a**), 0.1% HA (**b**), 0.3% HA (**c**); Night & Day: control (**d**), 0.1% HA (**e**), 0.3% HA (**f**). Figures present heat flow in function of temperature where two peaks (Proclear) or one peak (Night & Day) were observed.

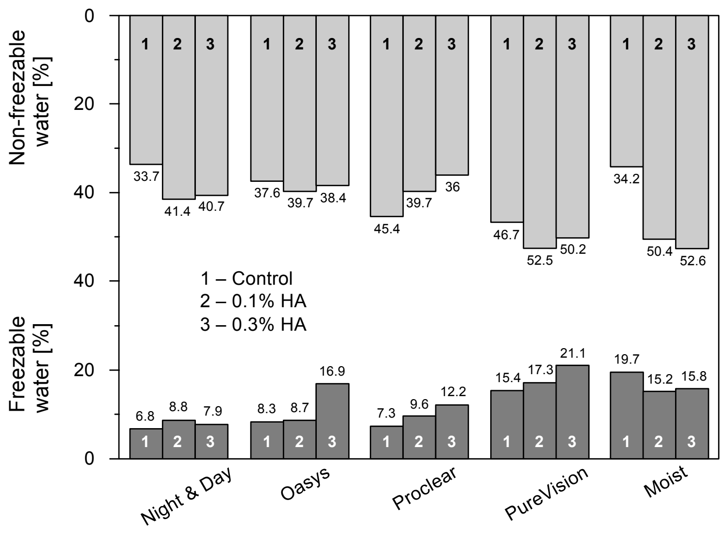

Fig. 6. Percentage of free (dark-grey) and bound water content (light-grey) in examined samples calculated from DSC measurements. EWC is the sum of free and bound water content.

Table 2. Total freezable (free and loosely bound) and non-freezable (bound) water content and free-to-bound water ratio for all examined lenses.

Materials	WC [%]	Freezable WC [%]	Non-freezable WC [%]	Free-to-bound water ratio
Night & Day, control	42.42	6.82	35.60	0.19
Night & Day, 0.1% HA	52.30	8.77	43.52	0.20
Night & Day, 0.3% HA	48.80	7.87	40.93	0.19
Oasys, control	45.84	8.27	37.57	0.22
Oasys, 0.1% HA	49.62	8.66	40.97	0.21
Oasys, 0.3% HA	56.06	16.93	39.13	0.43
Proclear, control	62.50	15.44	47.06	0.33
Proclear, 0.1% HA	70.41	17.29	53.13	0.33
Proclear, 0.3% HA	71.78	21.14	50.64	0.42
PureVision, control	52.65	7.26	45.38	0.16
PureVision, 0.1% HA	49.33	9.58	39.75	0.24
PureVision, 0.3% HA	48.17	12.16	36.01	0.34
Moist, control	54.04	19.65	34.39	0.57
Moist, 0.1% HA	67.01	15.21	51.80	0.29
Moist, 0.3% HA	69.07	15.83	53.23	0.30

– increase of free water and decrease of bound water content (PureVision),
– decrease of free and increase of bound water content (Moist).

The proportions of water are presented in Fig. 6. Free-to bound water ratio was calculated according to Eq. (4) and presented in Table 2.

4. Discussion

As follows from this study, sodium hyaluronate may cause changes in the material dehydration and water distribution. Initial mass of some examined lenses with HA was greater than control which indicates that HA induce water retaining abilities of lenses. This increase was not observed for Night & Day and PureVision whose surface was modified.

The DR parameters indicate that HA solutions influenced the dehydration pattern, although significant changes were observed only for Proclear. Previous studies found Proclear to be more resistant to dehydration (in vitro as well as *in vivo*) due to phosphorylcholine (PC) [27–29]. The presence of PC incorporated into material can also explain greater HA attracting ability. Due to small positive and negative charge of PC (dipole-like) in presence of HA, it might have created distribution of positive charge on the surface, which enabled to attract HA and in consequence enhance water retention.

The control lenses exhibited 3-phase dehydration pattern excluding Night & Day and Oasys where only two phases were observed. In all HA lenses three phases were noticeable. Our conclusion concerning relationship between water content and duration of phase I was consistent with previous results [6]. The increase of duration of phase I was observed for all lenses except PureVision. Nevertheless, as water content for PureVision did not increase, elongation of phase I was not expected. Interestingly, both HA solutions affected Night & Day and Oasys lenses – with HA phase I reached up to 12 min, while for control lenses phase I was not observed. It appears that increased water content generated prolongation of water retention in these lenses.

Study with the use of multipurpose solution containing HA (Biotrue) and contact lenses under conditions mimicking blinking exhibited that the ability of lenses to attract, retain and release depends on interaction between HA and contact lenses material characteristics [30]. After 14–15 h of soaking, the highest amount of attached HA was found for Night & Day (lotrafilcon A) and Air Optix (lotrafilcon B), the lowest for Pure Vision (balafilcon A) and Soflens (polymacon). Moreover after 20 h of rinsing lenses from I FDA group retained the most HA. However, in our study HA interaction was less apparent in case of these lenses. In present work Night & Day with HA reached higher water content (which can be explained by presence of HA and its water retaining ability), although due to high measurements variability the difference was not significant. PureVision indeed exhibited the lowest increase of water content which is in agreement with previous research. Nevertheless it is important not to overlook the fact that in our study the time of lens exposure on HA was shorter and probably these interactions should be considered more in terms of attaching than releasing HA.

In another study comparing wearing comfort and *ex vivo* dehydration of Air Optix with two multipurpose solutions (Biotrue and Complete Revitalens) the authors did not observe statistically significant differences in hydration with both solutions as well as with comfort and NIBUT [31]. They found it surprising considering that one of the solutions (Biotrue) contains HA which was expected to increase the wettability and hydration. Despite the fact that Air Optix attracts and retains relatively high amount of HA [30] it seems not to contribute to the higher hydration of the lens.

Higher heat flow through the samples was noticeable on melting endotherms which proves that HA–water–material interaction is present (Fig. 5). DSC melting endotherms for Oasys, Proclear and Moist presented two peaks supporting the discreet model of water in contact lens polymer [9]. Night & Day and PureVision presented only one around 0°C. Broad peak around –10°C appeared in lenses with HEMA component (Oasys, Proclear, Moist) (Table 1). As mentioned above, three models of changes of water types in lenses were observed.

Model 1 of HA–water interaction (increase of both types of water) was observed in case of non-ionic materials (Night & Day, Oasys, Proclear). As bound water creates direct hydrogen bonds with the polar groups of the polymer, HA might have replaced some molecules of water. High capacity for hydrogen bonding gives hyaluronan sponge-like characteristics [15] and in this way the increase of non-freezable water could be observed (models 1 and 2). Appearance of greater amount of free and loosely bound water seems to be due to HA attracted to lens interface with dispersion forces or dipole–dipole intramolecular interactions or trapped within the polymer [9, 30].

The ionic lenses (PureVision, Moist) exhibited visible and gradual change in free -to-bound water ratio. Model 2 (increase of freezable water and decrease of non-freezable water) for PureVision may indicate low affinity of lens matrix to create stronger bonding or even pushing away HA molecule soaked with water. That fact would be consistent with recent studies [30] and our findings from gravimetry in which both HA samples have not exhibited any change in DR values and even a slight decrease of phase I. PureVision seems to be the most resistant to interactions with HA molecule.

Interestingly, Moist showed decrease of free water for both solutions. Although overall percentage of water content enhanced significantly and non-freezable water amount increased which proves the ability of creating hydrogen bonding which theoretically is the driving force for lens attraction and retention of HA.

It appears that behavior of water, portions of free and bound water are strongly dependent not on concentration of HA, but on material properties. This relationship is not so simple as might be expected and since the number of samples used for the purpose of this experiment is low, any firm conclusions cannot be drawn from this part of study. Further research is necessary to explain these thermal interactions.

HA is an interesting molecule which has been proved in the recent works with incorporating HA into the lens matrix and its potential use in drug delivery [32]. It seems also important to understand the interactions with contact lenses in terms of eye-drop-like application. Hydration of the lens should be achieved preferably through the use of lubricating agents. However if their use does not link with any long-lasting interaction to lens matrix, the application seems to be less effective because of weaker attraction, thus faster release and drainage of the solution. Together with *in vivo* study, potential effectiveness of lubricating agent with different contact lens materials can be estimated, giving clinicians a useful tool in order to provide greater relief to symptomatic contact lens patients.

Our study shows that Proclear seems to be the most prone to attach HA molecules which affect changes in dehydration characteristics and water behavior in the polymer. PureVision might be considered as the most resistant to HA in terms of dehydration dynamics and water distribution. All measured parameters seem to be dependent more on material properties than HA concentration. Further studies are necessary to understand HA influence and its interactions with soft contact lens materials.

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