



# Characterization of albumin and hyaluronan-albumin mixed hydrogels

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## Abstract

Various hydrogels prepared from natural polymers have found great use in different industries, including, but not limited to, biomedicine. Recent trends in the material development focus on the preparation of hydrogels based on two or more biopolymers to combine their properties, which leads to better performance of a resulting material. In the present work, albumin and mixed hyaluronan-albumin hydrogels were characterized using various methods. The rheological properties of the samples varied based on the sample composition. The addition of hyaluronan to the albumin samples lowered the crosslink density of the hydrogels resulting in the lower complex modulus values and the higher equilibrium swelling ratio. Increasing the ionic strength of the sample shifted the albumin denaturation temperature of the water solutions to higher temperatures and suppressed the effect of hyaluronan on the denaturation. Scanning electron microscopy images showed that the microstructure of albumin samples significantly differs from the structure of mixed hyaluronan-albumin samples. The release profiles of a reference dye (methylene blue) showed, that the diffusion of the dye was rather slow. The released amounts of a dye from hydrogel into the buffer (physiological conditions model) varied from  $6.35 \pm 0.35\%$  up to  $9.78 \pm 1.42\%$  (after 96 h).

**Keywords** Albumin · Hyaluronan · Hydrogels · Rheology · Swelling · Diffusion

## Introduction

Hydrogels are described as 3D polymeric crosslinked network structures formed either by chemical or physical linking. (Hoffman 2012; Billiet et al. 2012; Ahmed 2015) It became clear that single polymer-based hydrogels cannot meet the requirements in both performance and properties. In order to improve the hydrogel performance, new materials based on two or more polymers have been developed in the last years. (Ahmed 2015) When considering using hydrogel as a biomaterial, the crucial property is its biocompatibility. (Anderson and Langone 1999) Hyaluronan (HA) and albumin are both biopolymers naturally occurring in the human body, which makes them perspective candidates for use in biomedicine.

*In vivo*, hyaluronic acid does not exist in the protonated acid form, but in the form of polyanion–hyaluronan (HA). (Necas et al. 2008) Its molecular structure is represented by repeating units of glucuronic acid and N-acetylglucosamine linked together through alternating  $\beta$ -1,3 and  $\beta$ -1,4-glycosidic bonds. (Necas et al. 2008; Fallacara et al. 2018) The roles of HA in the human body include maintaining elastoviscosity of connective tissues, tissue hydration, water transport, and various receptor-mediated roles. (Fallacara et al. 2018) Due to its specific interactions with cell receptors, HA is widely used as a component of the drug delivery systems. (Chen et al. 2018; Sharma et al. 2018) However, as a result of its strongly hydrophilic nature, a direct binding of hydrophobic drugs to HA is very complicated. To overcome these difficulties, HA has been chemically modified to form micelle-like structures capable of the conjugation of hydrophobic substances. (Mičochová et al. 2006) Although HA is completely biocompatible, it is known that biomaterials prepared from chemically modified natural polymers may still be immunogenic and cause inflammation and foreign body response *in vivo*. (Mooradian 2016) However, there is another possibility shows how to achieve the similar results—binding of hyaluronan to a protein capable of binding to a variety of hydrophobic drugs. (Lei et al. 2021).

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Serum albumins are the most abundant plasma proteins with a variety of functions and binding properties, e.g., solubilizing of long-chain fatty acids, binding bilirubin or therapeutic drugs (penicillins, sulfonamides, or benzodiazepines), or transport of metal ions in the blood. Due to its structural homology with the human serum albumin, bovine serum albumin (BSA) is the most extensively studied for its potential use in several areas including, but not limited to, biomedicine. BSA polymer chain consists of 583 amino acid residues, and it comprises 17 disulfide bridges and one free thiol group. (Peters 1985; Raja et al. 2015; Arabi et al. 2018) When heated, BSA undergoes conformational changes leading to the exposure of reactive sites resulting in the network formation. The overall physicochemical properties of the hydrogel are mostly determined by the nature of interactions and structural organization. (Arabi et al. 2018).

In their work, Lei et al. (2021) described the preparation of HA + BSA nanoparticles and some of the possible applications with promising results; however, the preparation of such systems still required chemical crosslinking and the use of organic solvents. In the previous work (Hájovská, et al. 2020), we have described the rheology of the mixed HA + BSA hydrogels in the form of semi-interpenetrating network; no chemical modification of polymers was involved. The ability of the HA + BSA complexes to bind hydrophobic substances was also proven.

The presented study now focuses on the further characterization of these materials. In this study, five representative BSA and HA + BSA hydrogels were characterized using oscillatory rheology, gravimetric studies of the swelling, thermal analysis (thermogravimetric analysis, differential scanning calorimetry and microcalorimetry) and scanning electron microscopy (SEM). Given the possible medical application of such systems, the release of a model substance from the hydrogel was monitored by the method of UV–Vis spectroscopy.

## Experimental

### Materials

Bovine serum albumin ( $\geq 98\%$ ) and the reference dye methylene blue were purchased from Sigma Aldrich. Sodium hyaluronate (HMW = 1500–1750  $\text{kg}\cdot\text{mol}^{-1}$  and LMW = 90–130  $\text{kg}\cdot\text{mol}^{-1}$ ) was purchased from ContiproPharma a.s., sodium chloride was purchased from Lach-Ner. Citric acid monohydrate, disodium hydrogen phosphate dihydrate, sodium hydroxide, hydrochloric acid, and disodium tetraborate octahydrate were purchased from Penta and used without further purification.

### Sample preparation

Before any use, HA was dried at 90 °C for 20 min. A calculated amount of dry polymer (BSA or HA) was weighed and added into the solvent (Milli-Q water or buffer) in the following manner: while stirring, small quantities of polymer were added into the beaker alternating with small amounts of solvent until the whole polymer weight was used. The resulting amount of solvent required to reach the desired concentration was calculated and added to the sample. The solutions were left stirring at 300 rpm for 24 h. The gels were prepared by placing each solution sample in a water bath at 80 °C for 24 h.

In the present work, five samples (see Table 1) were characterized for their rheological properties, shelf life, swelling, thermal behavior, structure (using SEM), and diffusion of reference dye. Based on the results of the previous study (Hájovská, et al. 2020), the representative samples with the overall polymer concentration of 13% w/v and the weight ratios of 1:12 (HA:BSA) in case of mixed samples were chosen. The citric acid/phosphate buffer with pH 7.0 and NaCl concentration of 0.15  $\text{mol}\cdot\text{dm}^{-3}$  was used.

### Rheological measurements

For all the rheological measurements, the AR-G2 rheometer (TA Instruments, New Castle, DE, United States) was employed. Parallel plate geometry, with the diameter of 25 mm and the measuring gap of 250  $\mu\text{m}$  (for solutions) or 1000  $\mu\text{m}$  (for gels) was used. The sample was loaded onto the bottom plate at the temperature of 25 °C and the top plate was then reduced to the appropriate measuring position. In order to avoid sample evaporation, a thin layer of silicone oil was pipetted onto sample edges. Prior to all measurements, the samples were left conditioning for five minutes. All the rheological measurements were performed in duplicate.

For all samples, the first measurement was the strain sweep test. The loss modulus  $G''$  and the storage modulus  $G'$  were monitored as a function of strain (from 0.01 to 100%) at the temperature of 25 °C and constant frequency

**Table 1** Table of prepared samples for characterization

Sample	Sample composition
A	BSA in water*
B	HA <sub>HMW</sub> + BSA in water*
C	HA <sub>LMW</sub> + BSA in water*
D	BSA in buffer**
E	HA <sub>HMW</sub> + BSA in buffer**

\*Milli-Q water, \*\*The citric acid/phosphate buffer (pH 7.0) and NaCl concentration of 0.15  $\text{mol}\cdot\text{dm}^{-3}$

of 1 Hz. As a result, a value of strain from the linear viscoelastic region was chosen for the subsequent measurements (2% for all samples).

Another test measured for all solutions and gel samples was the frequency sweep test, which monitored  $G'$  and  $G''$  as a function of oscillatory frequency (ranging from 0.1 to 100  $\text{rad}\cdot\text{s}^{-1}$ ) at 25 °C and constant strain 2%.

The shelf life study represented the observation of the stability of the rheological properties. The study was carried out by measuring the frequency sweep test of the gels in the intervals of 1, 3, 7, 14, and 28 days after sample preparation. During the study, samples were stored in the desiccator. One set of samples was stored at the laboratory temperature (23 °C) and the second set of samples was stored in the refrigerator (10 °C). As a result, the complex modulus  $G^*$  (at 10 Hz) change over time was evaluated. The  $G^*$  value (at 10 Hz) was calculated as an average from the two frequency sweep tests.

### Swelling properties study

The swelling properties of the gels were studied by the conventional gravimetric method. The gels were dried at 50 °C for 24 h. Then the resulted xerogels were weighed and immersed in Milli-Q water or buffer (pH 4.0, 7.0, 10.0). In the intervals of 1, 3, 7, 12 and 24 h, the swollen gels were taken out, the excess liquid was carefully dried from the surface and the gel samples weights were collected. According to Eq. (1), the swelling ratio ( $Q$ ) was then calculated as

$$Q = \frac{m_t}{m_0} \quad (1)$$

where  $m_t$  represents the weight of the swollen gel at a given time and  $m_0$  represents the weight of the dry gel.

### Thermal analysis

Thermogravimetric analysis (TGA) was performed using the TGA Q5000 analyzer (TA Instruments, New Castle, DE, United States). The gel samples were measured in the inert nitrogen ( $\text{N}_2$ ) atmosphere, in the temperature range 25–250 °C with a rate of 5 °C $\cdot$ min $^{-1}$ . Differential scanning calorimetry (DSC) was performed using the DSC Q200 analyzer (TA Instruments, New Castle, DE, United States). The temperature program was identical to the TGA measurement.

Microcalorimetric measurements were performed using TAM III (TA Instruments, New Castle, DE, United States). Solutions were pipetted into the vials in a volume of 1 mL (each sample in triplicate) and measured in the region of 25–80 °C, in which protein denaturation and crosslinking occurs. From the resulting curve of the normalized heat flow

vs. temperature, the denaturation temperature as a minimum of the peak was calculated.

### Scanning electron microscopy

Before any measurement, the hydrogels were dried at 65 °C for one hour. To ensure conductivity, the dried samples were sputter-coated with gold using Sputter Coater 7640 (Polaron Equipment, Ltd., Hemel Hempstead, UK) and imaged using EVO LS 10 scanning electron microscope (Carl Zeiss, Jena, Germany) with an operating voltage of 10 kV and with the magnifications of 500–5000.

### Release of a model substance

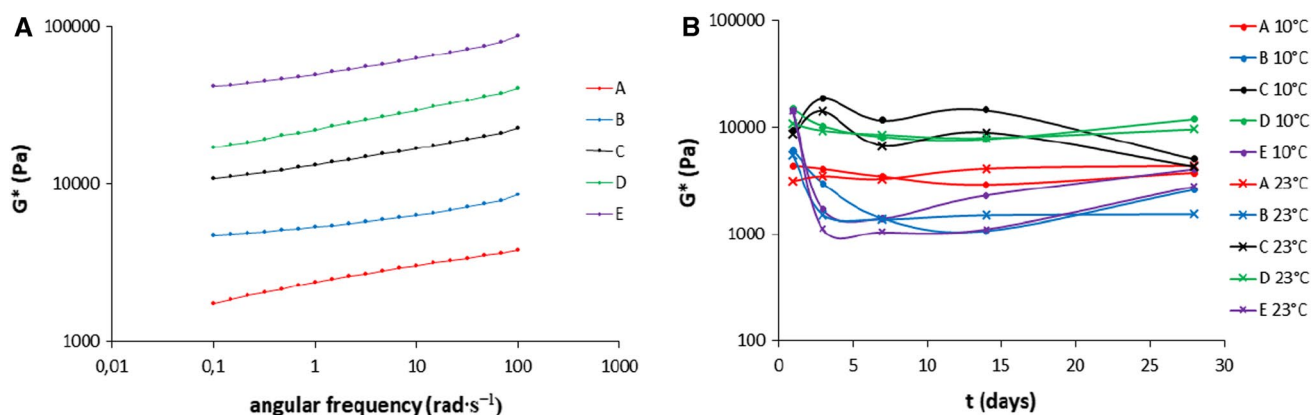
Possible drug release from the gel was simulated with a methylene blue as a reference dye. The gels were prepared with the methylene blue concentration of 1  $\text{g}\cdot\text{dm}^{-3}$ , every sample in triplicate. Based on the final volume of the sample, the specific weight of a methylene blue in each sample was calculated. The gels were then placed in 50 mL of Milli-Q water or buffer (pH 7.0,  $c_{\text{NaCl}} = 0.15 \text{ mol}\cdot\text{dm}^{-3}$ ). The release of the methylene blue was measured by UV–Vis spectrophotometer U-3900H (Hitachi, Ltd., Tokyo, Japan). The absorption spectra in the range 350–800 nm at the time intervals of 2, 4, 10, 24, 48, 72, and 96 h were acquired. The amount of a released methylene blue in the sample was evaluated based on the calculation from the calibration curve (absorption spectra at 665 nm). The resulting values were determined as an average from 3 measurements.

## Results and discussion

### Rheological measurements

Based on the previous measurements, the different properties of the samples prepared in water and buffer were expected as a result of alternating electrostatic interactions. These assumptions were confirmed by all measurements. As can be seen in Fig. 1a,  $G^*$  values of BSA sample in water are significantly lower compared to the sample of BSA in the buffer. The same trend can be observed for mixed samples.  $G^*$  values of the sample of  $\text{HA}_{\text{HMW}} + \text{BSA}$  in water are much lower compared to the sample of  $\text{HA}_{\text{HMW}} + \text{BSA}$  in the buffer. As it is obvious from the comparison of BSA and HA + BSA samples, the addition of HA itself leads to an increase of the  $G^*$  values. This behavior is consistent with the previous results, obtained from the study of the influence of the concentration and HA:BSA weight ratio (Hájovská, et al. 2020).

In general, the higher  $G'$  values (means also higher  $G^*$  values), the higher crosslinking density can be considered.



**Fig. 1** a: the plot of the complex modulus vs. angular frequency (frequency sweep); b: the plot of the complex modulus (at 10 Hz) vs. time for the gels stored at 10 °C (marked with ●) or 23 °C (marked with X)

(Okay 2010) The differences in the crosslinking density also lead to the different behavior or properties of the samples. These relationships are also discussed in the swelling properties study and SEM presentations.

During the shelf life study, different behavior of the samples was observed (see Fig. 1b). The rheological properties of the mixed hydrogels prepared with HA<sub>LMW</sub> or the single BSA gels were rather stable during the whole period of the experiment (4 weeks).

On the contrary, the  $G^*$  values of HA<sub>HMW</sub> + BSA hydrogels significantly dropped down during the first days after preparation. After this period, no substantial changes occurred. These results suggest that right after gelation the system HA<sub>HMW</sub> + BSA is not in the conformational state with the lowest energy. HA<sub>HMW</sub> with BSA in the solution is also known to form complexes behaving differently compared to single BSA solutions or HA<sub>LMW</sub> + BSA solutions. (Lenormand, 2010) Based on the equilibrium values of  $G^*$ , we can assume that the crosslinking density of HA<sub>HMW</sub> + BSA hydrogel is lower, compared to its analogs.

It was found out that the changes in the rheological properties were not significantly affected by the storage temperature (10 °C or 23 °C). However, a slightly more distinctive  $G^*$  decrease of the HA<sub>HMW</sub> + BSA samples at 23 °C was observed.

### Swelling properties study

The swelling of the gels was studied in the four different environments: non-ionogenic neutral (Mili-Q water), acidic (citric acid/phosphate buffer with pH 4.0), neutral ionogenic (citric acid/phosphate buffer with pH 7.0), and alkaline (borax/hydrochloric acid buffer with pH 10.0). HA + BSA gels are made up of polyelectrolyte chains; therefore the counter-ions are also present in the gel structure. Since the driving force of the swelling process is osmosis, it is clear

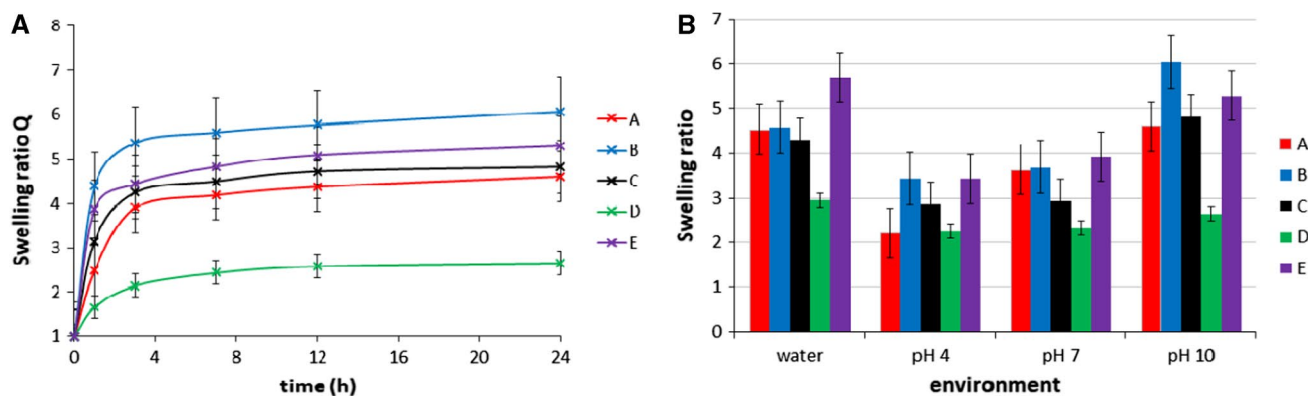
that the water molecules tend to permeate into the hydrogels. (Ganji, et al. 2010).

The results showed that the trend was the same for each sample in each environment. The example is shown in Fig. 2a. The most significant increase of  $Q$  can be observed during the first three hours of the experiment. After this period, only the subtle increase of  $Q$  was noticed. The equilibrium  $Q$  values ( $Q_{eq}$ ) reached after 24 h are summarized in Fig. 2b.

It is well known that gels prepared from ampholytes reach the lowest  $Q$  values in the environment with the pH = isoelectric point  $pI$ . (Park et al. 1998) All the results matched this assumption. The lowest  $Q_{eq}$  of every sample was observed at pH 4.0, which is the closest to  $pI_{BSA}$ . As the pH increased,  $Q_{eq}$  showed an increasing trend. By comparing the  $Q_{eq}$  reached in water with the one achieved in buffer pH 7.0, it is obvious that all gels swelled more in the water as a neutral non-ionogenic environment. This is because the osmotic pressure is driven by the concentration gradient, which is higher in the case of the gel-in-water system.

In general, the lower swelling ratio is, the higher crosslinking density we can consider. (Ganji, et al. 2010) From the pair of BSA samples in water and its analog prepared in the buffer, it was the sample of BSA in the buffer that swelled less in all four environments. These results indicate that the higher crosslinking density of this hydrogel and, therefore, higher elastic retraction force counteracting the network deformation. This conclusion also corresponds with the  $G^*$  values, which were higher for the BSA in the buffer sample, as mentioned in the section rheological measurements.

Other differences between the hydrogels prepared from water samples and those prepared from buffer samples can be discussed. Water samples exhibited the highest  $Q_{eq}$  in the buffer pH 10. This corresponds with the trend according to which increasing the pH leads to higher  $Q_{eq}$  value. On the



**Fig. 2** a: the plot of the swelling ratio vs. time, in the alkaline environment (pH 10); b: the plot of the equilibrium swelling ratio (after 24 h) for every sample in each environment

other hand, the gels prepared from buffer samples swelled the most in the water, not in the alkaline buffer. As it was described before, the driving force of the swelling is the osmosis driven by concentration gradient out and in the gel. The buffer gels do not have only the polymer chain counterions present in the network but also other low molecular ions increasing this gradient. The driving force is therefore much greater, resulting in higher  $Q_{eq}$  values.

For the pairs of BSA samples and their mixed  $HA_{HMW} + BSA$  analog, the following applies: as a result of lower crosslinking density (also stated as a result of shelf life study) the mixed samples exhibited higher  $Q_{eq}$  values. This behavior can also be enhanced by highly hygroscopic properties of  $HA_{HMW}$ . And finally, the difference between  $HA_{LMW} + BSA$  (lower  $Q_{eq}$ ) compared to  $HA_{HMW} + BSA$  can also be referred to higher crosslinking density of the  $HA_{LMW} + BSA$  sample. These conclusions also correspond with the previously discussed results of the rheological measurements.

### Thermal analysis

All TGA curves (supplementary materials–S1) were comparable for all the samples. Between 25 °C and approximately 100 °C, the significant drop down of the sample weight was observed as a result of water evaporation. The amount of lost weight ranged from 80.66% for the  $HA_{HMW} + BSA$  in the buffer to 86.98% for the BSA in the water sample. Up to 250 °C, no further changes were noticed. Similarly, the DSC curves (supplementary materials–S1) of the gels are very similar to each other. Up to 100 °C, an endothermic peak representing the water evaporation was observed. This trend fully corresponds with the TGA examination. Similarly, no further changes were observed.

Unlike TGA or DSC, which were used to describe the behavior of the crosslinked hydrogels, a microcalorimetric analysis was used to observe the heat-induced changes of the

polymer solutions, namely with a focus on protein denaturation, which precedes the crosslinking itself. The values of the denaturation temperature (calculated as a minimum of the endothermic peak during the slow heating) are presented in Table 2. Only minor differences between the samples were observed; however, the results are in accordance with the results from the rheological characterization (Hájovská, et al. 2020), which described the shift of the beginning of the gelation, as well as the gelation temperature, to higher temperature values as a result of higher ionic strength of the sample (see sample A vs. D and sample B vs. E). As can be seen from the comparison of the samples A vs. B, complex formation between HA and BSA increased the denaturation temperature; however, this trend is not obvious in the samples prepared in the buffer (B vs. E). Therefore, we can assume that the effect of the increased ionic strength plays a major role in influencing the BSA denaturation.

### Scanning electron microscopy

Morphology of the gels was studied using SEM. Images with the magnifications of 500 (supplementary materials–S2) and 5000 (see Fig. 3) were made. As we have already described before, there is an interesting difference between the mixed HA + BSA samples and the single BSA samples. The single BSA xerogels represent a homogenous, one-phase system.

**Table 2** Calculated values of denaturation temperature for each sample

Sample		Temperature (°C)
A	BSA in water	49.9 ± 0.1
B	$HA_{HMW} + BSA$ in water	51.4 ± 0.2
C	$HA_{LMW} + BSA$ in water	52.4 ± 0.1
D	BSA in buffer	52.2 ± 0.2
E	$HA_{HMW} + BSA$ in buffer	52.4 ± 0.3

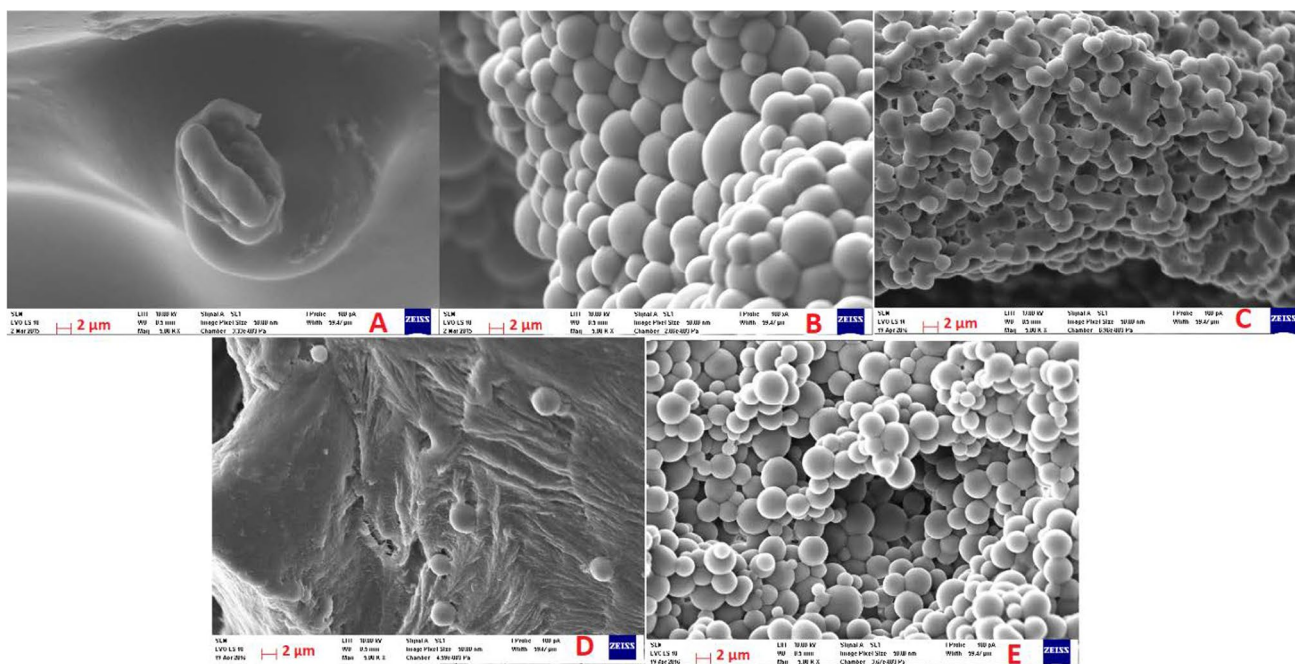


Fig. 3 SEM images of the xerogels (magnification 5000)

On the other hand, the structure of mixed HA + BSA samples is formed by spherical particles with the diameter in the range of 2–10  $\mu\text{m}$ . This difference can be explained with the HA + BSA complex formation in the solution leading to two-phase system formation. In the case of HA<sub>LMW</sub> + BSA (see Fig. 3c), spherical particles combine to form larger, more continuous formations. As can be seen in Fig. 3d and e, there is no significant difference between the microstructure of the BSA (HA + BSA) in water and BSA (HA + BSA) in buffer.

The results suggest that the morphology is highly affected by the HA addition to the BSA sample but also the molar mass of the HA plays a role in the resulting properties. These conclusions correspond with the data discussed in previous sections.

### Release of a reference dye

The subject of this experiment was the examination of the diffusion of a model substance (hydrophilic dye—methylene blue) into the water or buffer (pH 7.0,  $c_{\text{NaCl}} = 0.15 \text{ mol}\cdot\text{dm}^{-3}$ ) during the period of 4 days. The driving force of this process was the methylene blue concentration gradient in and out of the gel.

All the measurements proceeded similarly. The obtained data are presented in Fig. 4. The most significant increase of a released amount of a dye was observed during the first ten hours of the experiment. This period was characterized by the hydrogel swelling, and the dye was predominantly released from the sample surface, which is in direct contact

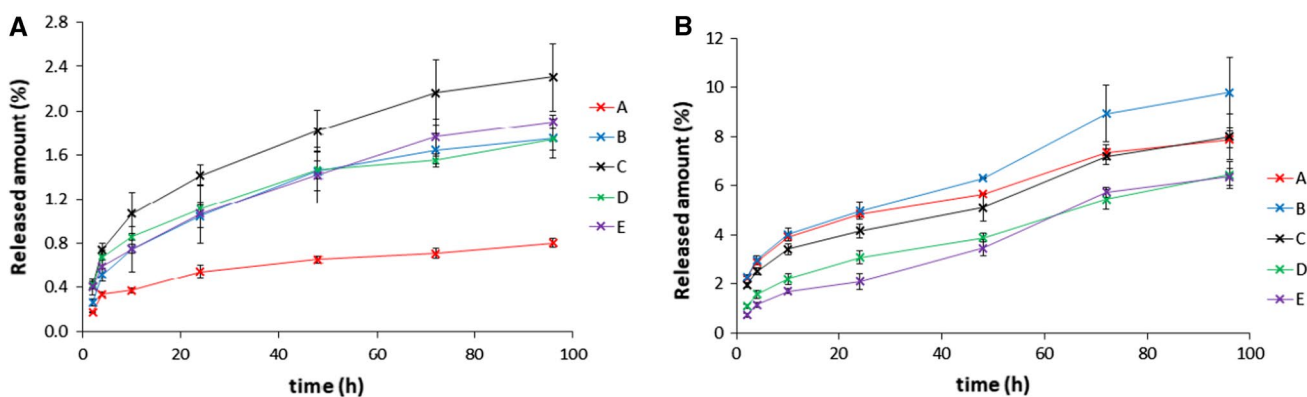


Fig. 4 the plot of the released amount of the reference dye (%) vs. time **a**: into the water; **b**: into the buffer

with the water (buffer). After this period, the reaching of  $Q_{eq}$  can be considered.

The released amount of a dye was slowly growing during the whole experiment. However, the values reached were rather low. Higher amounts of the dye (from  $6.35 \pm 0.35\%$  up to  $9.78 \pm 1.42\%$ ) were released into the buffer, compared with the release into the water (from  $0.80 \pm 0.04\%$  up to  $2.30 \pm 0.30\%$ ).

In case of release into the water environment (Fig. 4a), the presence of HA in the sample leads to an increase of the released amount of a reference dye. This behavior is more significant in case of hydrogels prepared solely in water (A vs. B and C), compared to the samples prepared in the buffer (D and E), which were comparable during the whole period of the experiment. Similar behavior was observed also in case of release into the buffer, where samples D and E reached approximately the same values. These results suggest that the presence of the low molecular ions in the sample, together with the controlled pH, suppresses the influence of the HA addition on the sample properties. These results also correspond with the microcalory measurements, where the values of denaturation temperature were influenced by the HA addition only in case of water samples; samples D and E reached comparable values (see Table 2). Based on the presented results, we can assume that the presence of low molecular ions affects the complex formation between BSA and HA, which in the end affects both, protein denaturation and also the interaction of the system with the ionic molecule of a reference dye.

Considering the chemical structure of methylene blue (its molecules have a positive charge when dissolved in water), an attractive interaction between the dye and the negatively charged polymer chains, partially immobilizing a dye in the gel network, can be considered. This behavior may be desirable in the cases when long-term release is required. If the slow diffusion is disadvantageous, it may be theoretically accelerated by decreasing the pH of the gel below the  $pI_{BSA}$  or by choosing a reference substance with different properties.

## Conclusions

The mixed hydrogels combining the properties of two or even more biopolymers represent an attractive material with the potential use in biomedicine. Here we presented the characterization of mixed HA + BSA hydrogels compared with the properties of its single BSA analog.

The addition of HA to the BSA sample seemed to decrease the crosslinking density of the hydrogel, resulting in the lower stable values of  $G^*$ . Another property directly related to crosslinking density is the swelling of the hydrogels. The mixed samples of HA + BSA reached higher  $Q_{eq}$

values than the BSA samples, and similarly, the water samples reached higher  $Q_{eq}$  values than their buffer analog. In general, the lowest  $Q_{eq}$  value of all examined gels was observed at pH 4.0, which is close to the  $pI_{BSA}$ .

The denaturation of the albumin was affected by the addition of HA into the sample, as well as the increasing the ionic strength. It was found out, that the thermal stability of the hydrogel samples was not affected by its composition. The only heat-induced change can be related to the water evaporation. On the other hand, the morphology differs significantly.

The release profile of model hydrophilic dye into the water and the buffer was measured. Considering the possible use of the hydrogels as some sort of a biomaterial, the buffer with its properties (pH and  $c_{NaCl}$ ) represented a basic model of physiological conditions. Release of a reference dye (methylene blue) to the buffer was characterized with the sharp increase at the beginning, followed with slow continuous release during the rest of the study. Slow release of a dye from a hydrogel can be considered both, disadvantage or advantage, depending on the use of the hydrogel material. Reaction kinetics may be influenced by changes in conditions such as pH, ionic strength etc. Such properties may be interesting for the applications as pH-sensitive drug carriers or in tissue engineering, to name a few. However, the real behavior of these samples should be the subject of further systematic study.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s11696-021-01853-y>.

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## Declarations

**Conflict of interest** On behalf of all authors, the corresponding author states that there is no conflict of interest.

## References

- Ahmed EM (2015) Hydrogel: preparation, characterization, and applications: a review. *J Adv Res* 6(2):105–121. <https://doi.org/10.1016/j.jare.2013.07.006>
- Anderson JM, Langone JJ (1999) Issues and perspectives on the biocompatibility and immunotoxicity evaluation of implanted controlled release systems. *J Control Release* 57(2):107–113. [https://doi.org/10.1016/S0168-3659\(98\)00178-3](https://doi.org/10.1016/S0168-3659(98)00178-3)
- Arabi SH, Aghelnejad B, Schwieger C, Meister A, Kerth A, Hinderberger D (2018) Serum albumin hydrogels in broad pH and temperature ranges: characterization of their self-assembled structures and nanoscopic and macroscopic properties. *Biomater Sci* 6(3):478–492. <https://doi.org/10.1039/c7bm00820a>

- Billiet T, Vandenhaute M, Schelfhout J, Van Vlierberghe S, Dubruel P (2012) A review of trends and limitations in hydrogel-rapid prototyping for tissue engineering. *Biomaterials*. <https://doi.org/10.1016/j.biomaterials.2012.04.050>
- Chen Y, Peng F, Song X, Jicheng Wu, Yao W, Gao X (2018) Conjugation of paclitaxel to C-6 hexanediamine-modified hyaluronic acid for targeted drug delivery to enhance antitumor efficacy. *Carbohydr Polym* 181(February):150–158. <https://doi.org/10.1016/j.carbpol.2017.09.017>
- Fallacara A, Baldini E, Manfredini S, Vertuani S (2018) Hyaluronic acid in the third millennium. *Polymers*. <https://doi.org/10.3390/polym10070701>
- Ganji F, Vasheghani-Farahani S, Vasheghani-Farahani E (2010) Theoretical description of hydrogel swelling: a review. *Iranian Polym J (english Ed)* 19(5):375–398
- Hájovská P, Chytil M, Kalina M (2020) Rheological study of albumin and hyaluronan-albumin hydrogels: effect of concentration, ionic strength, pH and molecular weight. *Int J Biol Macromol* 161(October):738–745. <https://doi.org/10.1016/j.ijbiomac.2020.06.063>
- Hoffman AS (2012) Hydrogels for biomedical applications. *Adv Drug Deliv Rev*. <https://doi.org/10.1016/j.addr.2012.09.010>
- Lei C, Xiu RL, Quan BC, You L, Jia LZ, Ling YZ, Tao Z (2021) Hyaluronic Acid and Albumin Based Nanoparticles for Drug Delivery. *Journal of Controlled Release*. Elsevier B.V. <https://doi.org/10.1016/j.jconrel.2021.01.033>
- Lenormand H, Deschrevel B, Vincent JC (2010) Chain length effects on electrostatic interactions between hyaluronan fragments and albumin. *Carbohydr Polym* 82(3):887–894. <https://doi.org/10.1016/j.carbpol.2010.06.011>
- Mlčochová P, Bystrický S, Steiner B, Machová E, Kooš M, Velbný V, Krčmář M (2006) Synthesis and characterization of new biodegradable hyaluronan alkyl derivatives. *Biopolymers* 82(1):74–79. <https://doi.org/10.1002/bip.20461>
- Mooradian, Daniel L. 2016. “Allografts and Xenografts in Soft Tissue Repair: Current Use and Future Trends.” In *Extracellular Matrix-Derived Implants in Clinical Medicine*, 41–62. Elsevier Inc. <https://doi.org/10.1016/B978-0-08-100166-0.00004-9>.
- Necas, J, L Bartosikova, P Brauner, and J Kolar. 2008. “Hyaluronic Acid (Hyaluronan): A Review.” *Veterinarni Medicina*. Vol. 53.
- Okay, O. 2010. “General Properties of Hydrogels.” *Hydrogel Sensors and Actuators* 6 (January 1970): 1–15. <https://doi.org/10.1007/978-3-540-75645-3>.
- Park HY, Choi CR, Kim JH, Kim WS (1998) Effect of PH on drug release from polysaccharide tablets. *Drug Deliv: J Deliv Target Therapeutic Agents* 5(1):13–18. <https://doi.org/10.3109/10717549809052022>
- Peters, Theodore (1985). “Serum Albumin.” *Advances in Protein Chemistry* 37 (C): 161–245. [https://doi.org/10.1016/S0065-3233\(08\)60065-0](https://doi.org/10.1016/S0065-3233(08)60065-0).
- Raja ST, Kumara TT, Mandal AB, Gnanamani A (2015) PH and redox sensitive albumin hydrogel: a self-derived biomaterial. *Sci Rep*. <https://doi.org/10.1038/srep15977>
- Sharma M, Sahu K, Singh SP, Jain B (2018) Wound healing activity of curcumin conjugated to hyaluronic acid. In vitro and in vivo evaluation. *Artif Cells, Nanomedicine Biotechnol* 46(5):1009–1017. <https://doi.org/10.1080/21691401.2017.1358731>

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