HYALURONAN BASED NANOPARTICLES FORMED BY ELECTROSTATIC INTERACTIONS

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Abstract

This work is focused on the preparation and characterization of nanoparticles. Particles were prepared from negatively charged polymer - sodium hyaluronate and oppositely charged polyaminoacid - polyarginine hydrochloride in water and 0.15 M sodium chloride. Due to the composition of nanoparticles from biodegradable materials, particles may be attractive for medical applications. Particles were studied using dynamic light scattering.

The second part of this work is focused on study of electrostatic interactions between negatively charged hyaluronan chains and simple amino acids (lysine and arginine) in water. These interactions were studied by means of calorimetry.

Keywords: Hyaluronan, amino acid, polyarginine, nanoparticles, dynamic light scattering, calorimetry

1. INTRODUCTION

Hyaluronan is a well-known and body's own negatively charged polysaccharide, which is of great interest in targeted drug distribution, especially anti-cancer, see, e.g., the monograph by Garg et al. [1]. In contrast, positively charged polyamino acids such as polyarginine consist of a body's own amino acids and their molecules are similar to molecules of peptides that are readily degradable in the body. Due to the opposite charge of the two polymers, the nanoparticles formed by the electrostatic forces between the polymers occur when they are mixed [2, 3]. Previous experiments have already confirmed the possibility of preparing such nanoparticles [2], which can be used as an instrument for targeted drug distribution. These experiments are followed by our work to simplify the preparation of nanoparticles and characterize them. For a more thorough examination of electrostatic interactions, we also studied the interactions between hyaluronan and simple amino acids.

2. EXPERIMENTAL PART

Hyaluronan (sodium salt) of molecular weight 9 kDa, 137 kDa and 1.39 MDa was purchased from Contipro Group s.r.o. Poly-L-argininehydrochloride of molecular weight ranging from 5 to 15 kDa was purchased from Sigma Aldrich. Polyarginine solutions of concentration 0.5 $g \cdot l^{-1}$ were prepared in deionized water (Purelab Flex, ELGA) and hyaluronan (137 kDa) solutions were also prepared in deionized water in concentrations 0.44; 1.67 and 2.67 $g \cdot l^{-1}$. Micro and nano particles were prepared by simply mixing hyaluronan solution of proper concentration and solution of polyarginine at room temperature in one-to-one volume ratio.

Size of the particles was measured in a several cases. In the first case, 1 ml of prepared solution with particles was taken and centrifuged for 30 minutes with 20 microliters of glycerol (Sigma Aldrich) at 16 000 g. In the next step supernatant was removed and particles with glycerol were mixed with 1 ml of deionized water. Particle size was measured in both the supernatant and the glycerol / water mixture. In the second case, the particles were measured after mixing the solutions of hyaluronan and polyarginine without further processing.

The influence of ionic strength on the particle size was tested by adding NaCl (Lach-Ner s.r.o.) to solutions formed by mixing solutions of hyaluronan and polyarginine. NaCl was added in such a quantity that the final concentration was 0.15 M.

L-lysine monohydrochloride and L-arginine monohydrochloride (Sigma Aldrich) were used for studying basic interactions between hyaluronan and amino acids.

3. REASULTS AND DISCUSSION

Nanoparticles of hyaluronan and polyarginine

Using the Zetasizer Nano ZS from Malvern Instruments, the correlation curves and particle sizes were measured in samples prepared in various ways. On the one hand, the particles prepared by centrifugation into the glycerol and the remaining particles in the supernatant, were measured. Also, particles prepared by simple mixing of solutions of hyaluronan (137 kDa) with different concentration with the polyarginine solution (0.5 g·l^{-1}), both before and after centrifugation, were also measured. The data obtained can be seen in **Table 1**.

Table 1 Measured values of particle sizes of nanoparticles composed of hyaluronan (Hya) and polyarginine (PArg) at different concentration ratios

Concentration (g·l^{-1}) Hya + PArg	Particle size (nm)			
	Hyaluronan + polyarginine + glycerol + centrifugation		Hyaluronan + polyarginine	
	Supernatant	Glycerol part	Centrifuged	Non-centrifuged
0.44 + 0.5	44 ± 10	70 ± 6	34 ± 1	59 ± 2
1.67 + 0.5	42 ± 2	69 ± 11	37 ± 1	68 ± 1
2.67 + 0.5	61 ± 2	91 ± 3	60 ± 1	90 ± 2

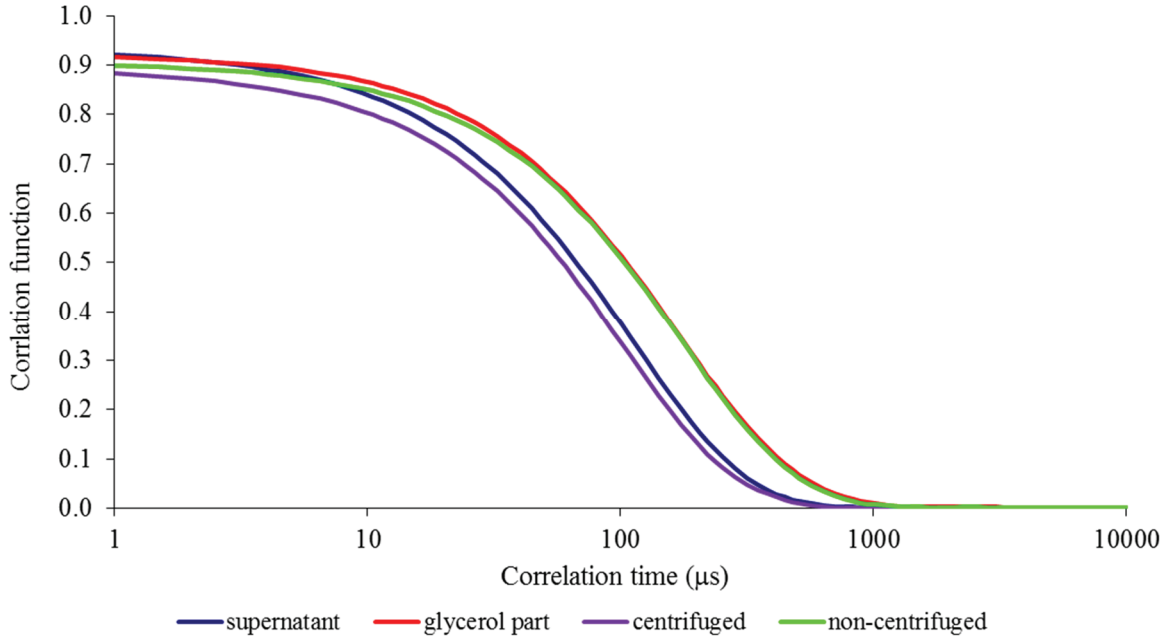

Figure 1 The hyaluronan-polyarginine nanoparticle correlation curves from hyaluronan solutions of 1.67 g·l^{-1}, and polyarginine solutions of 0.5 g·l^{-1}

From the graph in **Figure 1** it is apparent that samples labeled as supernatant and centrifuged have the same course of correlation curves and have very similar particle size values. Also, the samples labeled as glycerol and non-centrifuged have a very similar pattern of correlation curves and the corresponding particle size. With these simple experiments was found that the glycerol had no significant effect on the size of the centrifuged particles, therefore, in other experiments, particles were prepared with centrifugation but in the absence of glycerol in the Eppendorf tube.

Addition of NaCl to system hyaluronan-polyarginine

Unlike the previous study [2], where the effect of PBS on nanoparticles was studied, we decided to study nanoparticles only in 0.15 M NaCl. Samples of nanoparticles of the hyaluronan-polyarginine system were subjected to a stability test of NaCl addition. NaCl was added to the prepared solution of nanoparticles to a concentration of 0.15 M to simulate physiological environment. The influence of ionic strength on the particle size in the solution was determined by measuring particle size. By comparing the correlation curves shown in the graph in **Figure 2** we found that the addition of NaCl leads to a significant increase in particle size. This information was also confirmed by the values of the measured particle sizes listed in **Table 2**. After addition of NaCl to all solutions, all samples became immediately turbid. This fact was not described in the previous article [2].

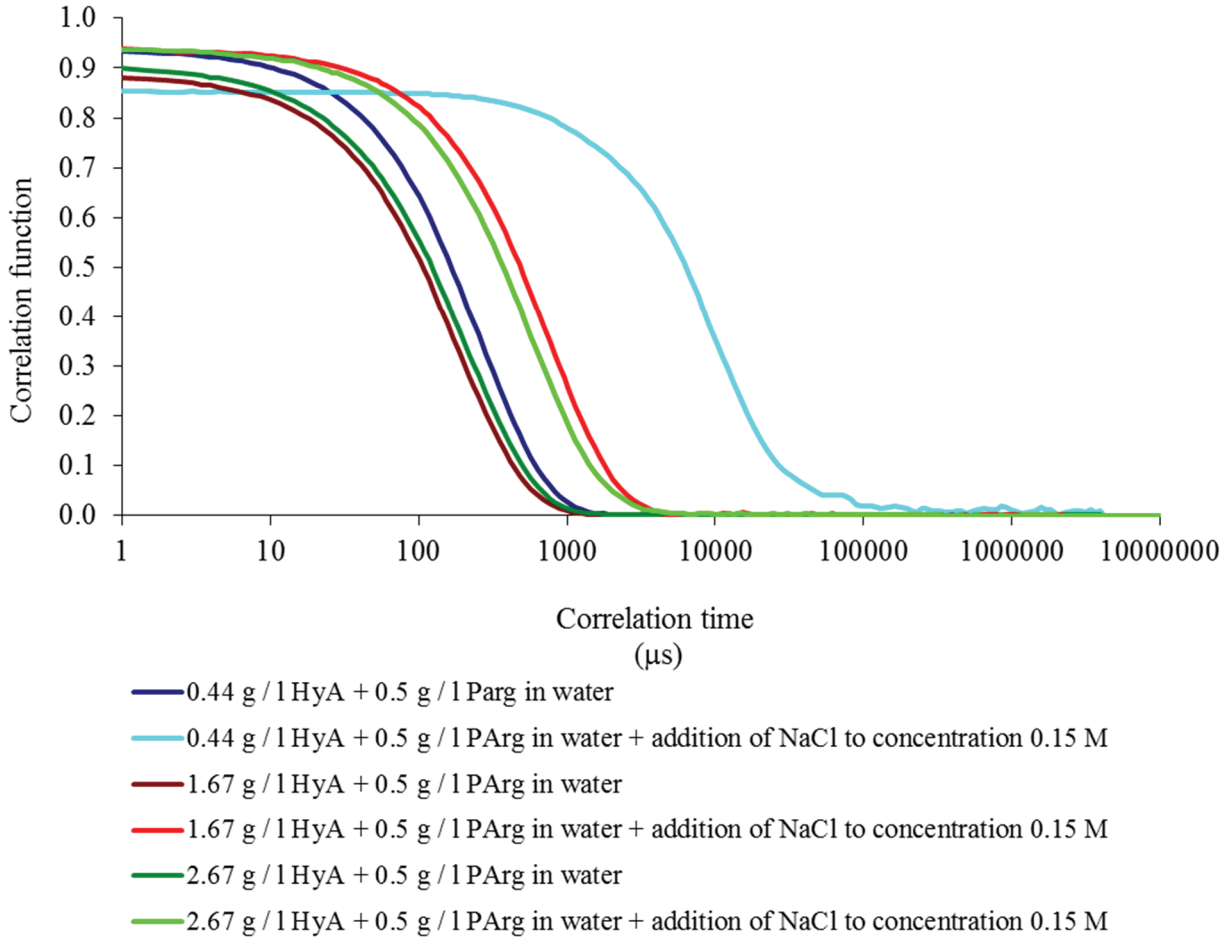

Figure 2 Correlation curves of hyaluronan nanoparticles at various concentrations and molecular weights of 137 kDa and polyarginine at a concentration of 0.5 g·l^{-1} and a molecular weight of 5-15 kDa, stability test by the addition of NaCl

Table 2 Measured particle sizes of the systems subjected to the NaCl stability test.

Concentration (g·l^{-1})	Particle size (nm)	
Hya + PArg	Before additon of NaCl	After addition of NaCl
0.44 + 0.5	193 ± 4	245 ± 78
1.67 + 0.5	145 ± 1	566 ± 14
2.67 + 0.5	166 ± 1	424 ± 6

Lyophilization

From the correlation curves shown in the graph in **Figure 3** we can observe that the lyophilization and subsequent dissolution of the samples resulted in an increase in the particle size. A larger increase of particle size occurred in the sample dissolved after lyophilization in 0.15 M NaCl solution. This information is also confirmed by the measured particle size values listed in **Table 3**. Samples after the lyophilization were dissolved for a relatively long time. The sample, which was dissolved in 0.15 M NaCl solution, had to be placed in an ultrasonic bath to dissolve it. Experiments with other concentrations (0.44 g·l^{-1}; 2.67 g·l^{-1}) of hyaluronan solutions were also performed. Similar results were achieved (results not shown).

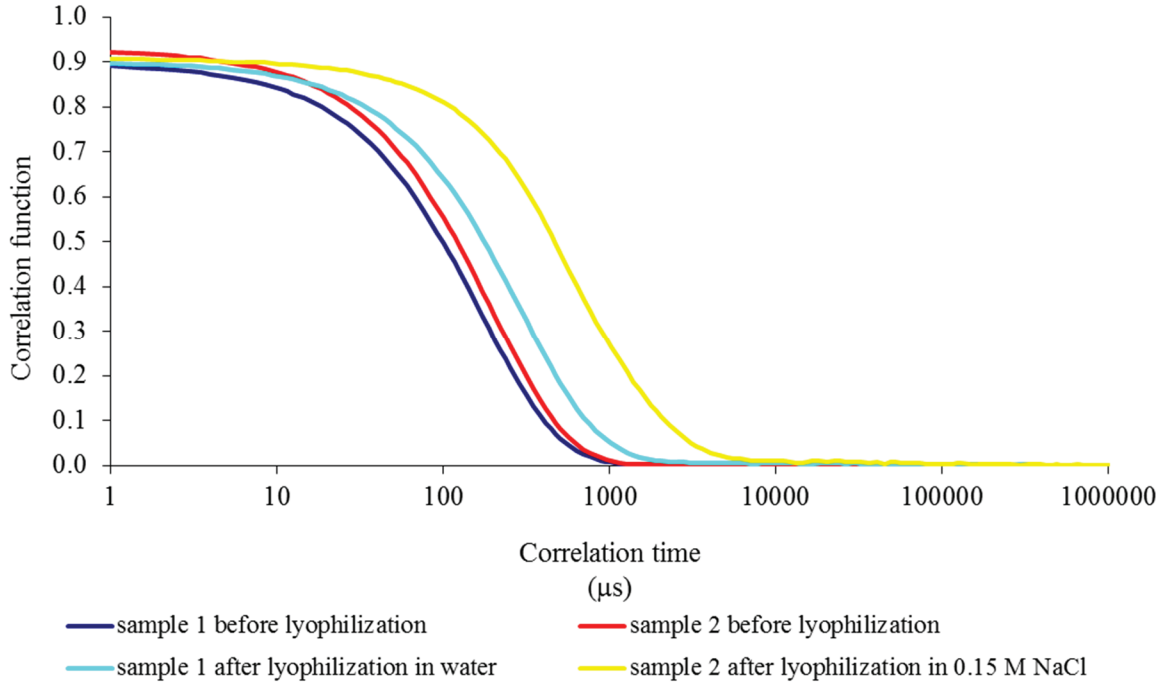

Figure 3 Correlation curves of 1.67 g·l^{-1} hyaluronan nanoparticles (137 kDa) and 0.5 g·l^{-1} polyarginine (5-15 kDa), lyophilized

Table 3 Particle sizes of the lyophilized systems

Concentration (g·l^{-1}) Hya + PArg	Volume ratio	After lyophilization dissolved in	Particle size (nm)	
			Before lyophilization	After lyophilization
1.67 + 0.5	1:1	Water	135 ± 3	226 ± 14
1.67 + 0.5	1:1	0.15 M NaCl	152 ± 5	523 ± 85

Hyaluronan and simple amino acid

In addition to interactions of hyaluronan with polyamino acids, hyaluronan (9 kDa and 1.39 MDa) interactions were also investigated with a simple amino acids arginine and lysine in their hydrochloride form. Interactions were studied primarily by nano ITC and high resolution ultrasonic spectroscopy, where amino acid solutions were titrated to hyaluronan solutions of various molecular weights. The concentrations were set such that the amount of charges, which carry the amino acids was nearly five times higher in the end of the titration than the number of charges in a solution of hyaluronate. From the results obtained, it was not possible to clearly determine if the interaction between hyaluronan and amino acid occurs (independently of the molecular weight of hyaluronan or type of amino acid). However, all performed titrations were endothermic.

4. CONCLUSION

Nanoparticles based on hyaluronan and polyarginine were prepared simply by mixing the appropriate solutions. It has been found that glycerol does not provide any advantage during the preparation over the preparation of nanoparticles without the glycerol. Particle stability was examined by lyophilization, re-dissolving and by addition of NaCl to simulate the ionic strength of the physiological environment. The results show that the particles in both cases increased their size, but prevent it from falling apart. It was also found that the addition of NaCl to the system causes the turbidity of the system. After lyophilization and redissolving or addition of NaCl, particle size values have exceeded the limits when it would be safe to use them as intravenous drug carriers, but other applications such as topical or mucoadhesive remain interesting.

For simple amino acids, all the interactions had the same endothermic course. It appears from the results that, rather than the formation of larger particles, the original hyaluronan structures present in the solution at the beginning of the experiment break down during the titration. It will be interesting therefore watch, how further experiments will be developed with polyarginine and polylysine oligomers and to find the boundary, where the oligomers begin to interact with hyaluronan forming nanoparticles.

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