Abstract

Physico-chemical interactions in hyaluronan-surfactant system result in nanoparticles formation which can be used in targeted delivery of hydrophobic active substances, drugs, in particular. These micellar aggregates have a core-shell like structure consisting of a hydrophobic inner core containing aggregated surfactant molecules and a hydrophilic hyaluronan shell layer.

The aim of our study was to prepare hyaluronic acid nanoparticles based on electrostatic interactions with oppositely charged molecules.

Particles were investigated using dynamic light scattering and Laser Doppler Velocimetry methods. Size distribution and zeta potential measurements were utilized to explore the formation of complex micelles. Formation of nanoparticles was achieved by addition of different volume ratio of negatively charged hyaluronan and positively charged surfactant. Particles were prepared in aqueous solution as well as in 0.15 M sodium chloride solution (physiological solution).

Polydisperse character of the hyaluronan-surfactant system was detected. Particle size in the water solution was in the range from 100 to 2 000 nm and size distribution of particles in the sodium chloride solution was in the range from 20 to 50 nm and from 200 to 1 000 nm.

Particles size distributions depend on type of solvent, molecular weight of hyaluronan and charge ratio of components of the hyaluronan-surfactant system. Aggregates formation is influenced by preparation of the system. Stability of the micellar particles depends on zeta potential value.

Keywords:
hyaluronan, surfactant, nanoparticles, dynamic light scattering, zeta potential

1. INTRODUCTION

Hyaluronan is a naturally occurring linear polysaccharide. It is a negatively charged biopolymer possessing one carboxylate group per disaccharide repeating unit (D-glucuronic acid and N-acetyl-D-glucosamine residues linked by β(1-4) and β(1-3) bonds). Despite the simple primary hyaluronan structure this molecule has very different biological effects depending on the molecule size and spatial arrangement. Hyaluronan can be found primarily in the extracellular matrix of all higher organisms, especially in connective tissues, synovial fluid, and eye vitreous and is produced by certain bacterial strains. Hyaluronan is therefore an attractive building block for new biocompatible and biodegradable materials that could have applications in drug delivery, tissue engineering, wound healing or surgery [1].

Hyaluronan cannot be directly used to carry nonpolar substances (hydrophobic drugs e.g. for fighting cancer) due to its highly hydrophilic character and large hydration shell. A combination of hyaluronan with surfactant may lead to formation of associates in which the surfactant hydrophobic domains solubilize hydrophobic substances and hyaluronan plays a role of biocompatible carrier and targeting agent [2-4].

Complex formation between a charged polymer and an oppositely charged surfactant occurs via two mechanisms. First, an ion-exchange process, second, at higher surfactant concentration, cooperative
binding. As the ratio of surfactant molecules to charged sites on the polyelectrolyte approaches unity, precipitation of the complex occurs due to charge neutralization of the polyelectrolyte and to the hydrophobic nature of the bound surfactants, which adopt a conformation in which their ionic headgroups are effectively removed from the solution. On addition of further surfactant, the insoluble polymer-surfactant complex redissolves. This is due to cooperative binding of surfactant molecules on the polymer chains, whereby hydrophilic micelles are formed [5, 6].

Polymer-surfactant interactions were subject of several previous studies and hyaluronan interactions with cationic surfactants were studied as a specific case of general polyelectrolyte-surfactant interactions to elucidate their phase behavior and physical causes of their interactions including the effect of electrolytes [7-9].

Dynamic light scattering method is one of many techniques for study of polyelectrolyte-surfactant interaction [10, 11]. Self-assembly behavior between hyaluronan and surfactant was studied using dynamic light scattering method, zeta potential and turbidity measurements [12]. Results confirmed that these systems are formed due to electrostatic interactions and it depends on molar ratio of components.

2. MATERIALS AND METHODS

Hyaluronan (as sodium salt of hyaluronic acid; HyA) at different molecular weights (in text below) was purchased from CPN, Ltd., Czech Republic. Cationic surfactant cetyltrimethylammonium bromide (CTAB) of the best available purity was purchased from Sigma-Aldrich, Czech Republic.

Stock solutions of hyaluronan and CTAB were prepared in aqueous solution and in 0.15 M sodium chloride solution. All stock solution were prepared by slow dissolution of powdered substances upon stirring and left stirred for 24 hours to ensure complete dissolution.

Formation of hyaluronan-surfactant nanoparticles was achieved by mixing of different volume ratio of negatively charged hyaluronan and positively charged surfactant. Different charge ratios of components were achieved by this. Preparation of hyaluronan-surfactant nanoparticles was performed using molecular weight of hyaluronan 106 and 1390 kDa and concentration 1 g·l⁻¹. At first hyaluronan was added dropwise into surfactant solution, afterwards surfactant was added dropwise into hyaluronan solution. The presence of turbidity in the sample volume was observed and it indicates the occurrence of micro- and nanoparticles in the system.

Titrations of substances were performed for investigation of interactions between hyaluronan and surfactant. Surfactant or hyaluronan was used as titrant. Isoelectric points (point of zero charge) were determined and compared. In the case of surfactant as titrant, concentration of surfactant titrant was 5 mM and hyaluronan concentration was 15 mg·l⁻¹. In the case of hyaluronan as titrant, concentration of hyaluronan titrant was 1 g·l⁻¹ and surfactant concentration was 1 mM. Initial volume of components was 10 ml. Hyaluronan was used at two molecular weights, 117 kDa and 1670 kDa.

Finally colloids were characterized by dynamic light scattering and laser Doppler Velocimetry methods. Size distribution (correlation function) and zeta potential measurements were utilized to explore the formation of complex micelles. All measurements were performed using Zetasizer Nano ZS (Malvern Instruments). All Zetasizer units provide a Z-average figure which is the intensity weighted mean hydrodynamic size of the ensemble collection of particles measured by dynamic light scattering. Z-averages of size distribution were compared and plotted.
3. RESULTS AND DISCUSSION

1.1 Behavior of hyaluronan in solution

According to autocorrelation function and related size distribution of sample we can conclude about quality of samples and interpret any problems with the samples. Hyaluronan aqueous solution is very polydisperse and this polydispersity increases with its molecular weight. Higher concentration of hyaluronan and use of 0.15 M NaCl solution as dissolving agent leads to form of more stable dispersion containing smaller nanoparticles. Low molecular weight and low concentration of hyaluronan in aqueous solution shows poor autocorrelation function owing to massive hydration shell creating a large “watery” random coil with refractive index close to surrounding environment. Particle size values of hyaluronan in aqueous solution are significantly deviate from average value. Addition of sodium and chloride ions into hyaluronan solution leads to contract of hyaluronan molecule and form more compact coil.

Zeta Potential is an important tool for understanding the state of the nanoparticle surface and predicting the long term stability of the nanoparticle. Zeta potential of hyaluronan solution in water is about −50 mV which means high degree of colloid stability of hyaluronan solution itself i.e., the solution is resist aggregation.

1.2 Preparation of nanoparticles

Formation of hyaluronan-surfactant nanoparticles is conditioned by volume ratio or more precisely charge ratio of mixing component, type of solvent, molecular weight of hyaluronan and concentration of surfactant stock solution. Aggregates formation is influenced by preparation of system, too. Different particles are formed if hyaluronan is added to surfactant solution or vice versa.

In the case of addition of low molecular weight hyaluronan to surfactant solution, local turbidity was observed already after addition of first 30 µl of hyaluronan solution. Turbidity increases with increasing added hyaluronan volume. Decrease of charge ratio at fixed surfactant concentration results in more intensive turbidity. Turbidity indicates presence of micro- and nanoparticles in the system. High molecular weight of hyaluronan causes more intensive turbidity than in the case of low molecular weight hyaluronan. It was found that formation of nanoparticles is influence by charge ratio of components and also initial concentration of surfactant.

The other way to formation of hyaluronan-surfactant micro- and nanoparticles is addition of surfactant into hyaluronan solution. In this case, formation of particles in the system occurred at higher volume (concentration) of added surfactant solution. If we add hyaluronan into surfactant solution, formation of aggregates occurs already after addition of first 30 µl of hyaluronan solution, i.e. at higher charge ratio of
components. This may be caused by excess of surfactant in the system. Micelles are present in the system and hyaluronan interact with them for creation of turbidity. When we add surfactant into hyaluronan solution, more intensive turbidity occurs after achievement of lower charge ratio. In this case, at first surfactant saturates surface of solution and afterwards micelles are formed. These micelles can interact with hyaluronan in the solution. It was found that the most intensive turbidity is created at slight charge excess of hyaluronan. Higher molecular weight of hyaluronan results in more intensive turbidity, too.

### 1.3 Titration

Titration was performed using hyaluronan or surfactant as titrant. In the case of surfactant as titrant, zeta potential increases mostly linearly during the titration. Initial zeta potential value of low molecular weight hyaluronan was about $$-40 \text{ mV}$$, for high molecular weight was about $$-20 \text{ mV}$$. Isoelectric point or more precisely point of zero charge shows concentration of surfactant when positive charge of surfactant equals to negative charge carrying on hyaluronan chain. It was found that measured isoelectric point does not correspond to theoretical isoelectric point supposing 100% dissociation of carboxylate groups of hyaluronan chain. Isoelectric point occurred at much lower surfactant concentration. It means that less than 100 % of hyaluronan carboxylate groups dissociate, practically less than 50 %. Measurements of size distribution of aggregates in the system were performed, too. It was found that size distribution of particle increases in point of zero charge or just before achievement of point of zero charge which means at slight charge excess of hyaluronan. The colloidal system exhibits in this point zero zeta potential and minimum stability.

When hyaluronan was used as titrant, initial zeta potential value of surfactant was about 20 mV. Zeta potential decreases linearly during the titration. Point of zero charge was found at lower hyaluronan concentration than theoretical value if 100% dissociation is assumed. It was found that only 20 % carboxylate group was dissociated. Increase of size distribution of aggregates before isoelectric point was observed, too.

![Additive titration graph for experiments using surfactant or hyaluronan as titrant.](image)

**Fig. 2** Additive titration graph for experiments using surfactant or hyaluronan as titrant. For experiments was used low molecular weight hyaluronan. Axis x indicates concentration of titrant; surfactant concentration (mM) for the case of surfactant as titrant and hyaluronan concentration (g·l\(^{-1}\)) for experiments in which hyaluronan was used as titrant. Dashed lines show found isoelectric points.
Fig. 3 Additive titration graph for experiments using surfactant as titrant (left) or hyaluronan as titrant (right). For experiments was used high molecular weight hyaluronan. Dashed lines show found isoelectric points.

4. CONCLUSION

Polydisperse character of hyaluronan was detected using dynamic light scattering measurements. It was found that the dissolution of hyaluronan in the sodium chloride solution gives us the smaller particle size in opposite to particle size obtained from the same concentrations of hyaluronan dissolved in water. Furthermore, it was found that systems consisting of cetyltrimethylammonium bromide and hyaluronan form particles in units of hundreds of nanometers, the particle size in sodium chloride solution were smaller than the same systems dissolved in aqueous solution.

Pilot titration experiments were performed to investigation of interaction between hyaluronan and opposite charged surfactant. Increase of aggregates size was observed near point of zero charge. The hyaluronan-surfactant system exhibits in this point zero zeta potential and minimum stability result in phase separation.

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LITERATURE


[6] WESLEY, Robin D., Terence COSGROVE, Laurie THOMPSON, Steven P. ARMES a Fiona L. BAINES. Structure of Polymer/Surfactant Complexes Formed by Poly(2-(dimethylamino)ethyl methacrylate) and Sodium Dodecyl


