STUDY OF NANOPARTICLES FORMED BY CATIONIC MICELLES AND HYALURONAN

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Abstract

Hyaluronan as negatively charged polyelectrolyte can interact with positively charged surfactant micelles via electrostatic interactions to form core-shell like nanoparticles. These aggregates can solubilize hydrophobic active substances and therefore are potential carriers in drug delivery applications. This research was conducted with the aim to prepare nanoparticles consisting of hyaluronan and cationic micelles and evaluate strength of electrostatic interactions between components.

Interactions of hyaluronan and surfactants in aqueous solution were investigated using turbidimetry method and stability of nanoparticles was studied using dialysis technique. Turbidimetric titration was chosen as indicator of the loss of intensity of transmitted light due to the scattering effect of particles associated from hyaluronan and surfactant in it.

Results of turbidimetry revealed that aggregates formation depends on hyaluronan concentration while surfactant concentration (above critical micelle concentration) affects interaction insignificantly. On the basis of turbidimetric results a system for dialysis experiments was selected. Dialysis experiments showed that a part of surfactant molecules is bound to hyaluronan chain and a rest of molecules diffuse to water through dialysis membrane and after a lapse of time the concentrations of the retentate and permeate were balanced. The system can be suitable for the preparation of targeted carriers of biologically active substances.

Keywords: hyaluronan, surfactant, nanoparticles, turbidimetry, dialysis

1. INTRODUCTION

Hyaluronan (refers to all physiological forms of hyaluronic acid, the most common of which is the sodium salt) is a polysaccharide found in the extracellular matrix, especially of soft connective tissues. It is a linear polymer built from repeating disaccharide units of β(1,4)-N-acetyl-D-glucosamine and β(1,3)-D-glucuronic acid. Despite the simple primary structure hyaluronan has substantial size heterogeneity in different tissues. Hyaluronan molecules have very different biological effects depending on the molecule size and spatial arrangement. Extensive studies on the chemical and physicochemical properties of hyaluronan and its physiological role in humans, together with its versatile properties, such as its biocompatibility, nonimmunogenicity, biodegradability, and viscoelasticity, have proved that it is an ideal biomaterial for cosmetic medical and pharmaceutical applications [1]. Hyaluronan and hyaluronan derivatives have been developed as topical, injectable and implantable vehicles for the controlled and localized delivery of biologically active molecules [2].

Hyaluronan has a highly hydrophilic character and large hydration shell and therefore it cannot be used to carry nonpolar substances. A combination of hyaluronan with surfactant should be used as possible variant to formation of aggregates in which the surfactant enables solubilisation of hydrophobic substances and hyaluronan plays a role of biocompatible carrier and targeting agent [3, 4].

Hyaluronan-surfactant interactions and physicochemical properties of this system were studied in several previous papers [5, 6, 7]. The aim of our work was to prepare hyaluronan-surfactant nanoparticles and study its properties and stability using turbidity measurements and dialysis technique.
1. MATERIALS AND METHODS

Hyaluronan (as sodium salt of hyaluronic acid; HyA) at molecular weights of 1635 kDa was purchased from CPN, Ltd., Czech Republic. Cationic surfactant Septonex (Carbethopendeciniumbromide) of the best available purity was purchased from GBNchem. Stock solutions of hyaluronan and Septonex were prepared in aqueous solution. All stock solutions were prepared by slow dissolution of powdered substances upon stirring and left stirred for 24 hours to ensure complete dissolution.

Turbidimetric titrations were carried out by adding hyaluronan solution at different concentration (0.05, 0.1, 0.3, 0.5 and 1 g/l) to the surfactant solutions of Septonex at concentration 3 mmol/l. Turbidity measurements, reported as absorbance A, were performed at 400 nm using Varian Cary 50 spectrometer equipped with a 1 cm path length fiber optics probe at 24 ± 1 °C.

Hyaluronan-surfactant system for dialysis experiments was formed spontaneously after mixing of components during 24 hours of stirring. Final hyaluronan concentration in these systems was 0.1 g/l and final concentration of surfactant was 3 mmol/l. Sample were transfer into dialysis membrane D-Tube™ Dialyzer Mega from Merck (MWCO 3.5 kDa). The membrane with the sample was immersed into pure Mili-Q water and left at rest at laboratory temperature for 48 hours. After 24 h and 48 h was taken the 3 ml of the sample from dialysis membrane and from aqueous solution outside of membrane. Surfactant concentrations inside and outside of the dialysis membrane were analysed using a method modified from Mahrou's et al. [8].

2. RESULTS AND DISCUSSION

2.1. Turbidimetry

First, effect of hyaluronan concentration was studied because of selection of enable system to dialysis experiments. Turbidimetric titration was chosen as an indicator of the loss of intensity of transmitted light due to the scattering effect of particles associated from hyaluronan and surfactant in it. During the titration experiments surfactant concentration decreases while hyaluronan concentration increases and a process of interactions of component can be study.

In Fig. 1 is shown results of turbidimetric titration experiments. Results revealed that aggregates formation (turbidity increasing) depends on hyaluronan concentration. In the case of hyaluronan concentration 0.05 and 0.1 g/l no significant increase of turbidity was observed. Hyaluronan concentration is insufficient for nanoparticles formation. If hyaluronan of concentration 0.3 g/l (or higher) is used as titrant, after a small addition of hyaluronan to surfactant solution turbidity slightly increases. This indicates a nanoparticle formation in the system. In the beginning, system is clear or slightly opalescent. With increasing hyaluronan concentration it becomes turbid and after a critical combination of component charges in the system a phase separation occurs.
Fig. 1 Turbidimetric titration results for the system with initial surfactant concentration of 3 mM and concentration of hyaluronan titrant 0.05, 0.1, 0.3, 0.5 and 1 g/l.

2.2. Dialysis

On the basis of turbidimetric measurements, system for dialysis experiments was selected. System consists of hyaluronan at concentration 0.3 g/l and surfactant at concentration 3 mmol/l. This surfactant concentration ensures that a micelle aggregates are presented in the systems. Critical micelle concentration of Septonex is reported as 0.80 ± 0.01 mmol/l [9].

In Fig. 2 is shown surfactant concentration determined by Mahrous method inside or outside dialysis membrane after 24 h and in Fig. 3 after 48 h. Surfactant concentration in blank sample (i.e. pure surfactant without hyaluronan) is shown in figures for comparison with the system with hyaluronan.

In Fig. 2 we can see that after 24 h surfactant concentration inside membrane is still higher than outside of membrane. This indicates that hyaluronan and surfactant create aggregates which are resisted to prompt disintegration after dilution of the system. For comparison is shown that in blank sample surfactant concentration is balanced on the both sides of the membrane after 24 h. Therefore we can declare that hyaluronan keeps surfactant micelles inside the membrane.
In Fig. 3 is shown the situation after 48 h. It is seems that surfactant concentration is almost balanced inside and outside of the membrane but is slightly higher than concentration in blank sample. This suggests that in the presence of hyaluronan in the system, a balance establishing is very complicated process and depends on all ions in the system. In permeate and in retentate there is a different composition probably because of
holding of some component on the surface of dialysis membrane and different permeation rates of constituent.

In consideration of obtained data, hyaluronan and surfactant create aggregates due to electrostatic interactions. These aggregates are not invariable but permanently reorganization occurs in the system. It was found that surfactant molecules are bound on hyaluronan by sufficient forces which resist prompt disintegration in molecules at least for 48 h. During this time hyaluronan slowly release surfactant molecules.

3. CONCLUSIONS

Turbidimetric titration and dialysis experiments provide information about hyaluronan-surfactant aggregates formation and about its stability. It was found that aggregates formation depends rather on hyaluronan concentration than surfactant concentration if it is above critical micelle concentration. It was found that hyaluronan-surfactant aggregates are sufficiently stable at least for 48 h and that slow release of surfactant molecules occurs.

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REFERENCES


