STUDY OF NANOPARTICLES FORMED BY CATIONIC MICELLES AND HYALURONAN

PILGROVÁ Tereza, PEKAŘ Miloslav

Brno University of Technology, Faculty of Chemistry, Institute of Physical and Applied Chemistry, Brno, Czech Republic, EU, xckrutisova@fch.vutbr.cz

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; therefore, they are potential carriers in drug delivery applications. The aim of this research was to prepare nanoparticles consisting of hyaluronan and cationic micelles and evaluate strength of electrostatic interactions between the components.

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

The results of turbidimetry revealed that aggregates formation depends on hyaluronan concentration while surfactant concentration (above critical micelle concentration) affects interaction insignificantly. Based on results, a system for dialysis experiments was selected. Dialysis experiments showed that a part of surfactant molecules is bound to hyaluronan chain and the rest of molecules diffuse to water through dialysis membrane. After a lapse of time the concentrations of the retentate and permeate were balanced. The system may 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 them is sodium salt) is a polysaccharide found in the extracelular matrix, especially of soft connective tissues. It is a linear polymer built from repeating disaccharide units of ß(1,4)-N-acetyl-D-glucosamine and ß(1,3)-D-glucuronic acid. Despite the simple primary structure, hyaluronan has substantial size heterogenity in different tissues. Hyaluronan molecules have very diverse 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; therefore, it cannot be used to carry nonpolar substances. A combination of hyaluronan with surfactant may be used as a possible variant of formation of aggregates in which the surfactant enables solubilisation of hydrophobic substances and hyaluronan serves as biocompatible carrier and targeting agent [3, 4].

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

2. 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 were 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. Samples were transferred 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 laboratory temperature for 48 hours. After 24 h and 48 h the 3 ml of the sample was taken 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 Mahrous et al. [8].

3. RESULTS AND DISCUSSION

3.1. Turbidimetry

First, effect of hyaluronan concentration was studied because of selection of enable system for 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 inside. During the titration experiments surfactant concentration decreases while hyaluronan concentration increases and a process of interactions of component can be study.

In **Fig. 1** are 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, solution turbidity slightly increases after a small addition of hyaluronan to surfactant. This indicates a nanoparticle formation in the system. In the beginning, system is clear or slightly opalescent. It becomes turbid with increasing hyaluronan concentration and a phase separation

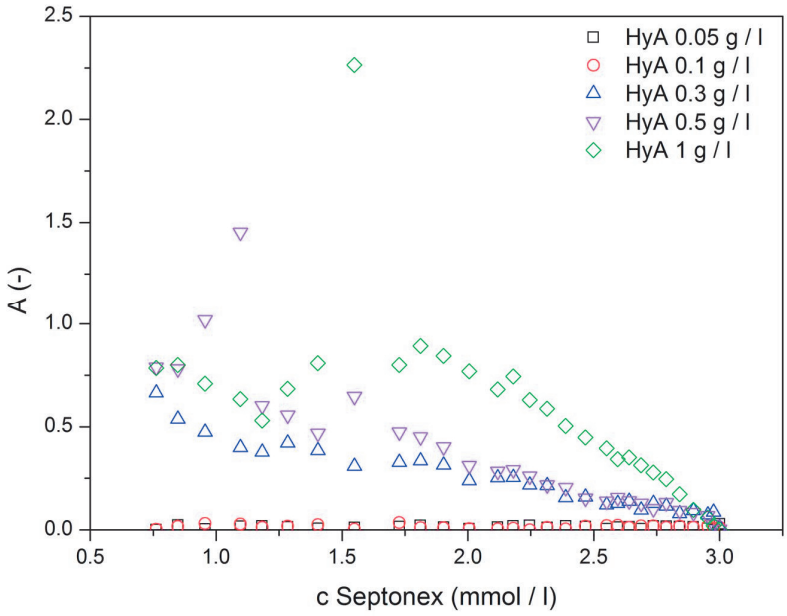

Fig. 1 Turbidimetric titration results for the system with initial surfactant concentration of 3 mM and concentration of hyaluronan titrant 0.05, 0.1, 0.3, 0.5 and 1 g / l

occurs after a critical combination of component charges in the system.

3.2. 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 a 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 surfactant concentration is balanced on the both sides of the membrane in blank sample after 24 h. Therefore we can declare that hyaluronan keeps surfactant micelles inside the membrane.

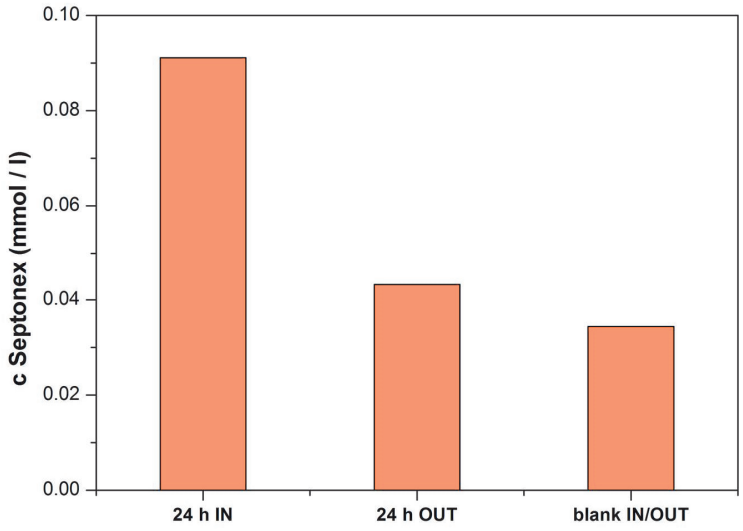

Fig. 2 Surfactant concentrations in and outside of dialysis membrane after 24 h

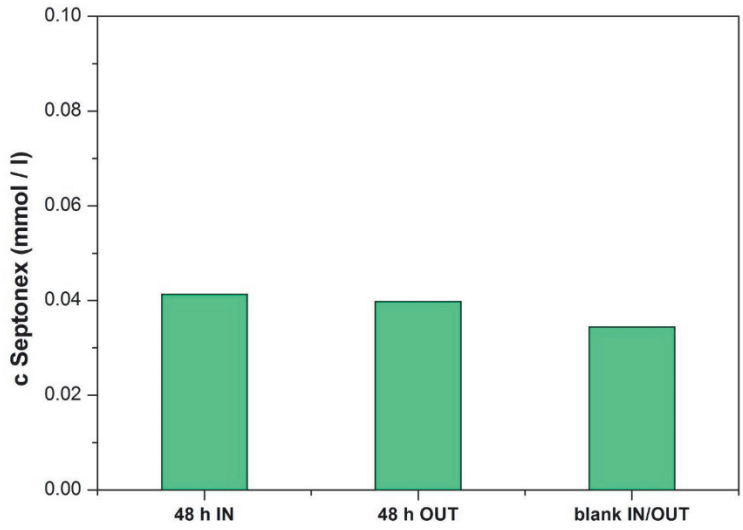

Fig. 3 Surfactant concentrations in and outside of dialysis membrane after 48 h

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 a balance establishing is very complicated process in the presence of hyaluronan in the system, and depends on all ions in the system. There is a different composition in permeate and in retentate 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.

4. 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. Hyaluronan-surfactant aggregates are sufficiently stable at least for 48 h and that slow release of surfactant molecules occurs.

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REFERENCES

[1] LIAO, Yong-Hong, Stuart A. JONES, Ben FORBES, Gary P. MARTIN a Marc B. BROWN. Hyaluronan: Pharmaceutical Characterization and Drug Delivery. *Drug Delivery*. 2015, 12(6): 327-342. DOI: 10.1080/10717540590952555. ISSN 1071-7544.

[2] LARSEN, Nancy E. a Endre A. BALAZS. Drug delivery systems using hyaluronan and its derivatives. *Advanced Drug Delivery Reviews*. 1991, 7(2): 279-293. DOI: 10.1016/0169-409X(91)90007-Y. ISSN 0169409x.

[3] OYARZUN-AMPUERO, Felipe A., Gustavo R. RIVERA-RODRÍGUEZ, Maria J. ALONSO a Dolores TORRES. Hyaluronan nanocapsules as a new vehicle for intracellular drug delivery. *European Journal of Pharmaceutical Sciences*. 2013, 49(4): 483-490. DOI: 10.1016/j.ejps.2013.05.008. ISSN 09280987.

[4] SAUEROVÁ, Pavla, Martina VERDÁNOVÁ, Filip MRAVEC, Tereza PILGROVÁ, Tereza VENEROVÁ, Marie HUBÁLEK KALBÁČOVÁ a Miloslav PEKAŘ. Hyaluronic acid as a modulator of the cytotoxic effects of cationic surfactants. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. 2015, 483(1): 155-161. DOI: 10.1016/j.colsurfa.2015.06.058. ISSN 09277757.

[5] XU, Jing, Huiyu BAI, Chenglin YI, Jing LUO, Cheng YANG, Wenshui XIA a Xiaoya LIU. Self-assembly behavior between native hyaluronan and styrylpyridinium in aqueous solution. *Carbohydrate Polymers*. 2011, 86(2): 678-683. DOI: 10.1016/j.carbpol.2011.05.006. ISSN 01448617.

[6] DASGUPTA, Satwati, Ranendu Kumar NATH, Kaushik MANNA, Ashish MITRA a Amiya Kumar PANDA. Physico-Chemical Studies on the Interaction of Bacterial Polysaccharide- Surfactant Aggregates with Special Reference to their Hydrodynamic Behavior. *Journal of Oleo Science*. 2014, 63(10): 1063-1075. DOI: 10.5650/jos.ess14080. ISSN 1345-8957.

[7] LINDMAN, Björn, Filipe ANTUNES, Saule AIDAROVA, Maria MIGUEL a Tommy NYLANDER. Polyelectrolyte-surfactant association-from fundamentals to applications. *Colloid Journal*. 2014, 76(5): 585-594. DOI: 10.1134/S1061933X14050111. ISSN 1061-933x.

[8] WURSTER, Dale Eric a Pornpen WERAWATGANONE. Influence of the polymer-micelle interaction on micelle-substrate binding. *Journal of Pharmaceutical Sciences*. 2010, 99(3): 1440-1451. DOI: 10.1002/jps.21889. ISSN 00223549.

[9] VOJTEKOVÁ, Mária, František KOPECKÝ, Olga GREKSÁKOVÁ a Jarmila OREMUSOVÁ. Effect of Addition of KX Type Electrolytes and Temperature on the Critical Micellar Concentrations of 1-Cetylpyridinium and Carbethopendecinium Bromides. *Collection of Czechoslovak Chemical Communications*. 1994, 59(1): 99-105. DOI: 10.1135/cccc19940099. ISSN 0010-0765.