STABILITY OF THE COMPLEX NANOPARTICLES OF NEGATIVELY CHARGED HYALURONAN AND CATIONIC SURFACTANT

Tereza KRUTIŠOVÁ, Miloslav PEKAŘ

Brno University of Technology, Faculty of Chemistry, Institute of Physical and Applied Chemistry, Purkyňova 464/118,61200 Brno, Czech Republic, xckrutisova@fch.vutbr.cz

Abstract

Hyaluronan and surfactant interact due to relatively strong electrostatic interactions between negative charge on hyaluronan chain and cationic parts of surfactant molecules. These interactions lead to complex nanoparticles formation which can be used in targeted delivery of hydrophobic active substances. 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 and study stability of these particles under different conditions.

Time and temperature stability, effect of ionic strength, effect of dilution of the system and effect of molecular weight of hyaluronan were investigated using fluorescence spectroscopy and dynamic light scattering methods. Fluorescent probe pyrene was selected for spectroscopy experiments because of its unique sensitivity to polarity of the medium. Particle size distributions of aggregates were obtained by dynamic light scattering measurements.

Fluorescence and dynamic light scattering experiments indicate that hyaluronan-surfactant nanoparticles are influenced by a whole range of aspects, such as temperature, ionic strength of solution and storage time while molecular weight of hyaluronan affect these system at least. Dilution experiments show that our complex nanoparticles are insufficiently stable and are not able to maintain hydrophobic active substances in the inner core of aggregate after dilution of the system.

Keywords:

hyaluronan, surfactant, nanoparticles, fluorescence spectroscopy, pyrene, dynamic light scattering

1. INTRODUCTION

Hyaluronan (refers to all physiological forms of hyaluronic acid, the most common of which is the sodium salt) is the simplest glykosaminoglycan (a class of negatively charged polysaccharides) and a major constituent of the extracellular matrix. It is a linear, unbranched, alternating polymer composed of two monosaccharides - ß(1,4)-N-acetyl-D-glucosamine and ß(1,3)-D-glucuronic acid. Despite the simple primary hyaluronan structure this molecule has very different biological effects depending on the molecule size and spatial arrangement. The chain length of hyaluronanu varies from 0.2 to 10 MDa, with the most common sizes ranging from 2–5 MDa. Hyaluronan can be found primarily in the extracellular matrix of all higher organisms, especially in connective tissues, synovial fluid, and eye vitreous and is a scaffold secreted by cells that surrounds them *in vivo*. 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, 2].

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 [3, 4].

Polymers and surfactants can be used as mixtures bringing new and strong advantages into the field of drug delivery. The characteristics of these drug vehicles may be tuned varying different parameters such as size and type of the hydrophobic alkyl chain of the surfactant, the nature and size of the polar head group of the surfactant, concentration, salt content, temperature, pH and presence of co-solutes. Surfactants micelles are highly cooperative, organized molecular assemblies of amphiphiles, yet dynamic in nature. Micelles can be of any desired charge type and can adopt different shapes and internal packing, depending on the chemical structures of the constituent monomers, temperature and the ionic strength of the medium [5]. Polymers are used in drug delivery due to their efficiency as stabilizers, their capacity to form gels and to control the rheology, even at low concentrations, and also, in special cases, analogous to surfactants, their capacity to form self-assembled stable structures. Polymer-surfactant systems present several different properties from the individual behavior of polymer or surfactant systems. Of particular interest, it is the fact that polymer-surfactant associative mixtures are capable of forming concentrated complexes/nanoparticles upon dilution. If on one hand the degradation or disruption of surfactant and polymer systems has particular interest in some cases, on the other hand dilution or degradation of the drug vehicle in the body fluids is not desired before a particular site of action is reached, keeping particle integrity [6].

The aim of our work was to prepare hyaluronan-surfactant system and study its stability under different conditioned. This should provide information about behavior of these systems in physiological environment after application of the system and especially about effect of storage on properties and stability of these systems.

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 Septonex of the best available purity was purchased from GBNchem.

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

Hyaluronan-surfactant nanoparticles were formed spontaneously after mixing of components during 24 hours of stirring. Final hyaluronan concentration in these systems was 15 $mg \cdot l^{-1}$ and final concentration of surfactant was 0.05 or 0.07 $mmol \cdot l^{-1}$, respectively. Hyaluronan was used in two molecular weights (117 or 1635 kDa).

Time and temperature stability, effect of ionic strength and effect of dilution of the system were investigated using fluorescence spectroscopy and dynamic light scattering methods. Fluorescent probe pyrene was selected for spectroscopy experiments because of its unique sensitivity to polarity of the medium. Fluorescence emission spectra were recorded on Steady State Spectrofluorometer from HORIBA Scientific. Excitation wavelength of pyrene was 336 nm. Intensity ratio of first and third vibronic peaks (EmPI - the pyrene polarity index or the ratio of fluorescence intensities at 373 and 383 nm) is a reflection of the micropolarity in the vicinity of pyrene and it is used to detect the localization of pyrene in the system. EmPI for polar environment is in the range of 1.25–2.0 and indicate pyrene in aqueous solution. When pyrene is in the micellar solvent, EmPI is about 1.0–1.5. This value indicates that pyrene molecule is inside micelle, in palisade layer of micelle. EmPI for absolutely nonpolar solvent is about 0.5–0.6 (for example hydrocarbon solvent or micelle inner core). Pyrene experiments were also evaluated by a ratio of fluorescence intensities at 470 nm (emission maximum of excimer) and first vibronic peak (at 373 nm). This ratio is referred to as Ex:Mo. It is the indicator of probability of excimer formation in the system [7, 8].

Particle size distributions of aggregates were obtained by dynamic light scattering measurements using Zetasizer Nano ZS (Malvern Instruments). All measurements were performed at laboratory temperature.

The hyaluronan-surfactant system was prepared in the same way for all experiments. In the case of time experiments, system was measured once a week over one month. All samples were storage at 4 °C and before measurement were equilibrate to laboratory temperature. In the case of effect of ionic strength, 121 µl of saturated solution of sodium chloride (6.2 mol/l) was added to the sample so that final concentration of sodium chloride was 0.15 mmol·l^{-1} and sample volume was almost unchanged. Hyaluronan at concentration of 15 mg·l^{-1} and relevant molecular weight was used for dilution of the systems that were diluted ten times. Temperature stability was study in the temperature range of 10–50 °C.

3. RESULTS AND DISCUSSION

3.1 Time stability

According to polarity index EmPI and probability of excimer formation expressed as Ex:Mo ratio we can conclude about presence and quality of hydrophobic domains in the system. Based on this, it was found that polarity of pyrene molecules environment change insignificantly. First day after system preparation pyrene is localized in hydrophobic inner core of aggregates, for example minimicelles associated with hyaluronan chain, and excimer formation probability is relatively high. In the course of time, pyrene is distributed more evenly and thereby excimer formation in reduced. System becomes more stable with respect to system arrangement.

From the point of view of particle size distribution it was found that distributions change only negligibly and systems are monodisperse. Particle size is mostly in the range of 100 – 250 nm.

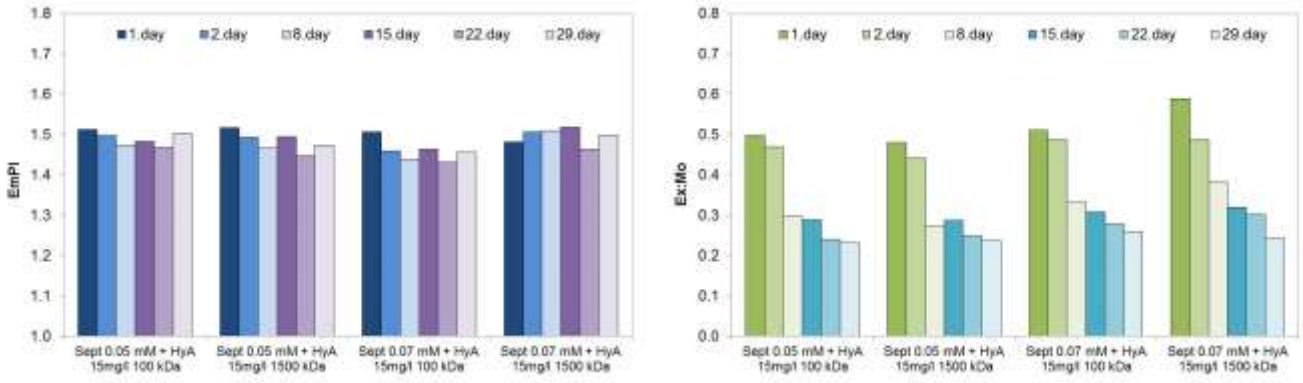

Fig. 1 Dependence of polarity index EmPI and Ex:Mo ratio on storage time of Septonex-hyaluronan samples

3.2 Temperature stability

Quality of hydrophobic domains of hyaluronan-surfactant aggregates was study in the range of 10–50 °C. It was found that systems of various compositions behave differently. In the most cases polarity index EmPI increase with increasing temperature. This change shows that aggregate shell become less compact and enables water penetration into hydrophobic inner core of aggregates and thus EmPI index increases.

Probability of excimer formation decreases with increasing temperature. This corresponds with rearrangement of the system and pyrene localization change or aggregates structure changes or aggregates disruptions.

3.3 Effect of ionic strength

Presence of low molecular weight electrolyte in the system results in decrease of excimer formation probability. It was found that system is not stabilized because of hydrophobic solute presence and addition of small amount of NaCl in the system causes significant changes in the system structure.

It was found that system polydispersity increases after NaCl addition due to elimination of electrostatic repulsion between aggregates in the system. In the case of aqueous solution, system is monodisperse and particle sizes are in the range of 100–150 nm. After NaCl addition (in the physiological solution) system becomes polydisperse and particle sizes are in the range from 30 nm to 5500 nm with a different distribution.

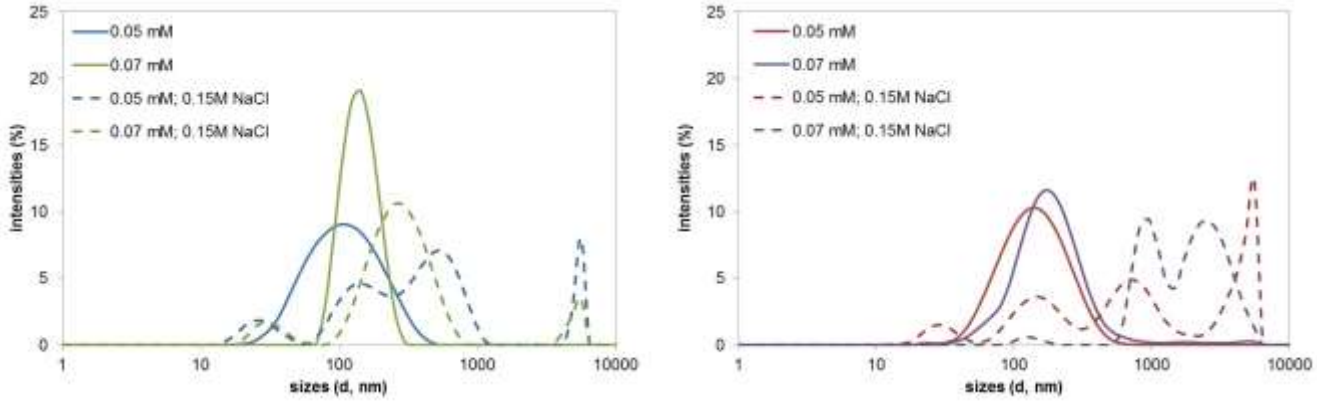

Fig. 2 Particle size distribution of Septonex-hyaluronan system (left – 116 kDa, right – 1635 kDa) in aqueous solution (continuous line) and in physiological solution (broken line).

3.4 Effect of dilution

Minimicelles associated with hyaluronan chain exist in the system before dilution and it provide hydrophobic domains for solubilization of hydrophobic fluorescent probe. Pyrene molecules can localized in the inner core of aggregates and thus form excimer. After dilution of the system polarity index EmPI increases and probability of excimer formation decreases significantly. These changes are caused by disintegration of the aggregates in the system. These changes show that these complex aggregates are not sufficiently stable to maintaining of hydrophobic solute inside aggregates. These systems are very dynamic and respond to even the slightest change in the composition of the system.

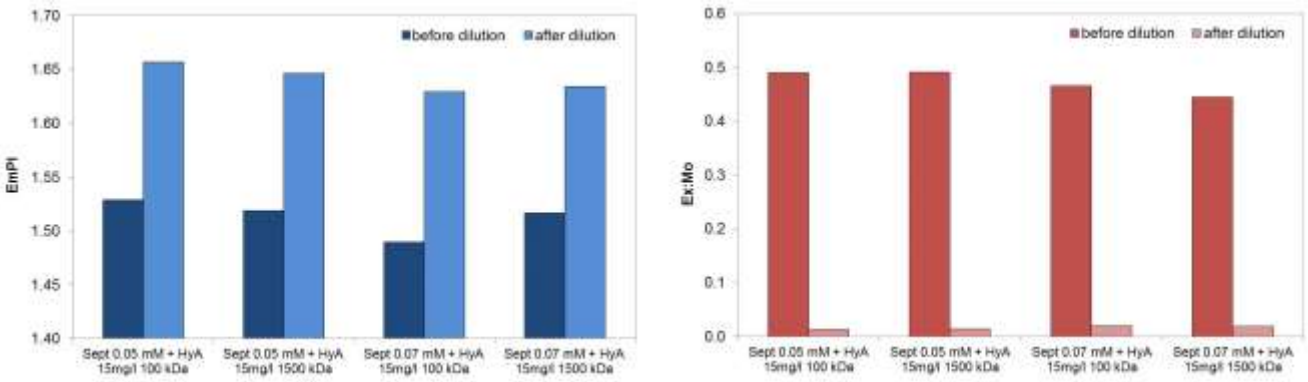

Fig. 3 Polarity index EmPI and probability of excimer formation (Ex:Mo) before and after dilution of systems

4. CONCLUSION

Fluorescence spectroscopy and dynamic light scattering methods provide a lot of information about stability of hyaluronanu-surfactant systems. It was found that these systems are influenced by a whole range of aspect, such as ionic strength of solution, temperature and storage time of final systems. Molecular weight of hyaluronan and a small different between component concentration affect stability of these system at least. Significant changes of component concentration represent by dilution experiments show that our complex nanoparticles are insufficiently stable and are not able to maintain hydrophobic active substances in the inner core of aggregate after dilution of the system.

5. ACKNOWLEDGEMENT

This work is supported by project Materials Research Centre at FCH BUT- Sustainability and Development, REG LO1211, with financial support from National Programme for Sustainability I (Ministry of Education, Youth and Sports) and COST action CM1101, project No. LD12068.

6. LITERATURE

[1] LAURENT, Torvard C. a J. Robert E. FRASER. Hyaluronan. *The FASEB Journal*. 1992, roč. 6, č. 7, s. 2397-2404.

[2] LAURENT, Torvard C., Ulla B. G. LAURENT, J. Robert FRASER a J. Robert E. FRASER. Functions of hyaluronan. *Annals of the Rheumatic Diseases*. 1995, č. 54, s. 429-432. DOI: http://dx.doi.org/10.1002/9780470513385.ch2.

[3] MALMSTEN, Martin. Surfactants and polymers in drug delivery. New York: Marcel Dekker, c2002, vii, 348 p. ISBN 08-247-0804-0.

[4] CHAVANPATIL, Mahesh D., Ayman KHDAIR, Yogesh PATIL, Hitesh HANDA, Guangzhao MAO a Jayanth PANYAM. Polymer-surfactant nanoparticles for sustained release of water-soluble drugs. Journal of Pharmaceutical Sciences. 2007, vol. 96, issue 12, s. 3379-3389. DOI: 10.1002/jps.20961. Available from: http://doi.wiley.com/10.1002/jps.20961

[5] CHAUDHURI, Arunima, Sourav HALDAR a Amitabha CHATTOPADHYAY. Organization and dynamics in micellar structural transition monitored by pyrene fluorescence. *Biochemical and Biophysical Research Communications*. 2009, vol. 390, issue 3, s. 728-732. DOI: 10.1016/j.bbrc.2009.10.037. Dostupné z: http://linkinghub.elsevier.com/retrieve/pii/S0006291X09020014

[6] COELHO, Edited by Jorge. *Drug delivery systems advanced technologies potentially applicable in personalised treatment*. Dordrecht: Springer, 2013, s. 35-85. ISBN 9789400760103.

[7] GLUSHKO, V., M.S.R. THALER a C.D. KARP. Pyrene fluorescence fine structure as a polarity probe of hydrophobic regions: Behavior in model solvents. *Archives of Biochemistry and Biophysics*. 1981, vol. 210, issue 1, s. 33-42. DOI: 10.1016/0003-9861(81)90160-0. Dostupné z: http://linkinghub.elsevier.com/retrieve/pii/0003986181901600

[8] DONG, Dao Cong a Mitchell A. WINNIK. THE Py SCALE OF SOLVENT POLARITIES. SOLVENT EFFECTS ON THE VIBRONIC FINE STRUCTURE OF PYRENE FLUORESCENCE and EMPIRICAL CORRELATIONS WITH E T and Y VALUES. *Photochemistry and Photobiology*. 1982, vol. 35, issue 1, s. 17-21. DOI: 10.1111/j.1751-1097.1982.tb03805.x. Dostupné z: http://doi.wiley.com/10.1111/j.1751-1097.1982.tb03805.x