EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

Experiment Report Form

	Experiment title: Inhalable Hyaluronate – Dexamethasone formulation for COVID-19 ventilated patients	**Experiment number**: MD-1253
Beamline: ID02	**Date of experiment**: from:04/12/2020 to:06/12/2020	**Date of report**:
Shifts: 6	**Local contact(s)**: Narayanan Theyencheri, Michael Sztucki	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

The experiment took place remotely. The experimentalists at the beamline were the local contacts.

Del Favero Elena, Università di Milano, BIOMETRA Dept.

Cantù Laura, Università di Milano, BIOMETRA Dept.

Camara Candelaria Inés, Universidad Nacionale de Còrdoba, Argentina

Ricci Caterina, Università di Milano, Bioscienze Dept.

Report:

The specific aim of this proposal was to characterize by SAXS the structure of Hyaluronic-Dexamethasone nanoparticles (HA-Dex Nps). HA-Dex Nps were design to treat patients on ventilators in intensive care units, with COVID-19 infections.

Both molecules, Dexamethasone (Dex, a synthetic anti-inflammatory) and Hyaluronic acid (HA, highly negative polyelectrolyte) have a high anti-inflammatory activity. It has been reported that Dexamethasone can suppress the over reactions of the immune system of patients with COVID-19 infection and restrain inflammation. High molecular weight HA can be selectively captured by macrophages and is able to polarize theses cells toward and anti-inflammatory phenotype. We propose a drug delivery strategy based on lung administration of the HA-dexamethasone formulation in order to increase the drug concentration in the target pulmonary tissue and in the alveolar macrophages and reduce the systemic exposure.

Different batches of HA-Dex Nps were characterized in order to evaluate the reproducibility of the synthesis procedure. Also, the Nps dispersed on diverse solvents (water, phosphate buffer and phosphate in presence of NaCl) were studied. By last, we studied the interaction of HA-Dex Nps with mucus model to evaluate mucoadhesion/mucopenetration, stability or structural modifications.

Figure 1, shows the SAXS intensity profiles of the different batch of HA-Dex Nps dispersed in phosphate buffer (PB; pH: 7.4). These profiles show that the global structure of the HA-Dex Nps batch are very similar. The profiles have a slope value of -1 at high *q* value indicating the existence of a rigid rod structure, probably due to the presence of HA in solution or adsorbed on the HA-Dex NPs. In the range of *q* lower than 0.1 the slope changes abruptly to a value of -4, indicating the presence of large particles with a solid Dex core with a defined interface. In this range of *q* values, SAXS profiles of the diverse synthesis show difference in intensity possible due to a variation in the size of the particles. In all intensity profiles two well defined peaks are present at high q values, q1 = 4.71nm^{-1} and q2=5.41 nm^{-1}, corresponding to the characteristic distances of the microcrystalline structure of dexamethasone[1].

The Nps were also studied in differens solvents, as can be seen in Figure 1b. When the HA-Dex Nps are dispersed in water an additional structure factor peak is present, as indicate the black arrow in Figure 1b. A similar structure factor was reported for semiflexible polyelectrolyte as HA in solution [2].

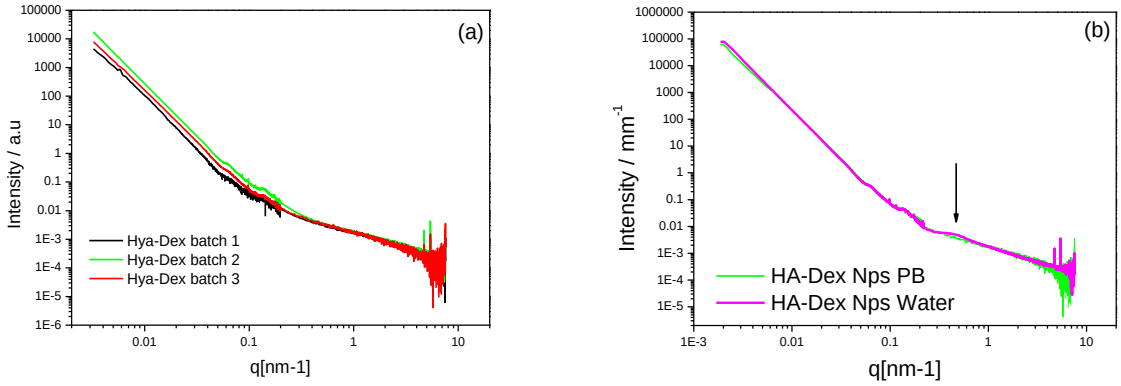

Figure 1**:** SAXS intensity profiles for HA-Dex Nps (a) differents batchs in PB and (b) batch 2 in PB and water.

HA-Dex / Mucin interaction. Also, we studied the behavior of HA-Dex nanoparticles when dispersed in mucin of different concentration, particularly 0.5% p/v and 1% p/v. The aim was to study the mucoadhesion/mucopenetration, stability or structural modifications of the particles. Figure 2 shows SAXS spectra of free HA-Dex nanoparticles (green), Mucin (red), HA-Dex in mucin (black). The interaction between mucin and HA-Dex particles was analyzed by comparing the obtained spectra after subtracting the corresponding contribution of mucin (blue spectra), with the profile of the free HA-Dex particles (green spectra). When the nanoparticle concentration was 2.5mg/ml the subtraction didn´t recover the original HA-Dex scattering spectra (expected result if mucin and HA-Dex did not interact), suggesting a modification of the external nanoparticle structure in the presence of Mucin. A similar behavior was found for Mucin concentration of 1% p/v.

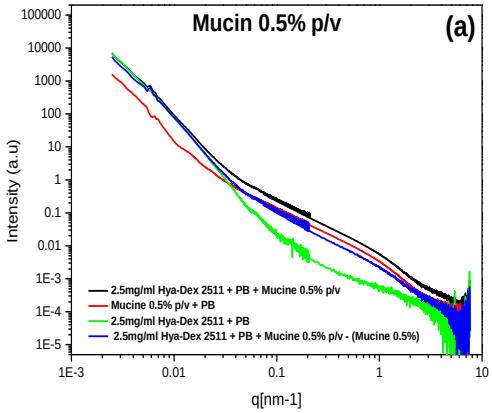

Figure 2: SAXS profile for 2.5mg/ml HA-Dex Nps batch 2; Mucin 0.5% p/v.

[1] K. Florey, G.A. Brewe, E.M. Cohen, D.E. Guttman, S.M. Olin, G.J. Papariello, B.Z. Senkowski, Federick. Tishler, Analytical Profiles of Drugs Substances, 1973rd ed., Academic Press, London, 1973.

[2] M. Villetti, R. Borsali, O. Diat, V. Soldi, K. Fukada, SAXS from polyelectrolyte solutions under shear: xanthan and Na-hyaluronate examples, Macromolecules. 33 (2000) 9418–9422. https://doi.org/10.1021/ma000971z.