Context.

Chondroitin Sulfate (CS) is a sulfated glycosaminoglycan found in the brush region of aggrecan in the extracellular matrix of cartilage. It gives it compressive strength thanks to the repulsion of its negative charges; CS is given as a drug for osteoarthritis treatment or as a food supplement.

Chain length: 20 - 60 nm. Chain width: ~ 1 nm.

Aim.

Study CS behavior, specifically chains flexibilty, characterized by the persistence length $L_p$, in ionic environments of different valences and concentrations ($c_i$) to improve the understanding of its biological properties.

Relate the obtained results with polyelectrolyte theories: Oosawa-Manning condensation and Odjik-Skolnick-Fixman (OSF) $L_p$. Predicts a linear relationship between $L_p$ and inverse $c_i$ for single ion environments.

Methods.

• Establishment of a protocol optimizing the observation of CS in various ionic environments.
• Investigation of the effect of ionic environment on CS.

Purification

Transformation into Na- and Ca-forms (CS-Na and CS-Ca) by ion-exchange and dialysis.

Solutions preparation

Dilution to yield solutions with a polymer concentration of 1 µg·mL$^{-1}$ and $c_i$ (of Na and Ca, respectively) ranging from 0 to 100 mM:

<table>
<thead>
<tr>
<th></th>
<th>CS-Na</th>
<th></th>
<th>CS-Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>$c_i$</td>
<td>0</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5</td>
<td>10</td>
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<tr>
<td></td>
<td>15</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>100</td>
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</tr>
</tbody>
</table>

Observation by Atomic Force Microscopy (AFM)

Samples prepared on mica modified with (3-aminopropyl)triethoxysilane allowing polymer adsorption on substrate. High resolution scans (scan size: 5 µm, scan rate: 0.1 Hz, 5120 pixels) obtained for each sample.

Data treatment with FiberApp

FiberApp is a software developed in the laboratory of Food and Soft Materials to track polymers coordinates and extract data to study polymers physics. Challenging step since polymers are very small and difficult to observe and distinguish from each other.

Contour length and average height extraction

Contour length (for both ion-forms and all $c_i$) : from 23 to 173 nm. Difference with values in litterature (20 to 60 nm) might be due to error resulting from challenging tracing.

Average height (both ion-forms and all $c_i$) : from 0.13 to 0.61 nm. Variations with values from litterature (0.35 to 0.65 nm) are associated with changing polymer-substrate interactions.

Persistence length extraction

Using 2D WLC model for semi-flexible chains. Important fluctuations most probably due to tracing. Relationship between $L_p$ and inverse $c_i$ is not linear.

Conclusions.

• OSF theory not followed in these two cases.
• Condensation of counterions not clear; further experiments necessary.
• $L_p$ of CS-Na higher than CS-Ca’s at $c_i < 5$ mM.