

# Extraction of hyaluronic acid and chondroitin sulphate from marine biomass and their evaluation as bioactive polymers in ocular carrier formulation

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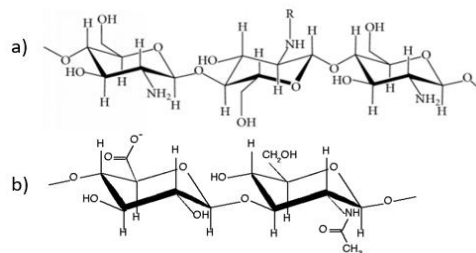
## Summary

Hyaluronic acid (HA) and Chondroitin sulfate (CS) are extracted and recovered from marine biomass for their application in new carrier formulations of drug delivery systems to treat Dry Eye Disease (DED). Marine biomass, including codfish bones and sardine heads and bones, is a low cost and renewable source. The extraction of the biopolymers will be done using Deep Eutectic Solvents (DES) as novel and green solvents. DES and the isolated biopolymers will be physically and chemically characterized. In addition, bioassays will be also performed to assess the toxicity, muco-adhesion, anti-inflammatory and anti-oxidant activity to ensure their safe application on DED treatment.



## State-of-the-art

HA and CS have shown to be effective compounds in the treatment of DED. Their conventional extraction techniques are highly complex and time-consuming, as they include the use of organic solvents, enzymes and detergents to break the cellular structures of the tissues from which HA and CS are extracted. The extraction by enzyme digestion requires different solvents and expensive enzymes, such as papain, pepsin, pronase and trypsin. On the other hand, the use of organic solvents, including chloroform, make the technique highly toxic. Therefore, the application of a green methodology that based on the use of alternative solvents is strongly recommended. In this study, DES are applied as low cost, novel and green solvents to extract HA and CS. Different combinations of DES at specific ratios will be evaluated to obtain the most suitable solvents to dissolve HA and CS from marine sources, at optimal extraction conditions. Hence, marine biomass is used as the source for the biopolymers, as it is a cheap and highly abundant renewable raw material.



Bianchera et al. (2014), Onken (2001)

Chemical Structure of a) Chondroitin Sulfate (R=H or COCH<sub>3</sub>),  
 b) Hyaluronic acid



## Techniques

The green novel technique is based on the use of different combinations of DES to isolate the biopolymers. The extraction conditions (time, temperature, components ratio) will be studied to optimize the process. DES and the isolated biopolymers will be characterized to assess the physicochemical properties using HPLC, FTIR, SEM, NMR, DSC and TGA. The stability, toxicity, muco-adhesive property, anti-inflammatory and anti-oxidant activity are studied to ensure the safe application of the biopolymers in carrier



## Task description

- Choose the most suitable marine raw materials based on the amount of HA and CS detected and apply different DES combinations to extract HA and CS, and optimize the extraction conditions.
- Regenerate HA and CS from the biopolymer-DES mixture or use biopolymers-DES as a therapeutic solution.
- Physicochemical characterization of regenerated HA and CS.
- *In vitro* cytotoxicity, mucoadhesion and anti-inflammatory activity of regenerated HA and CS or biopolymers-DES systems to assess the safe application of the compounds.