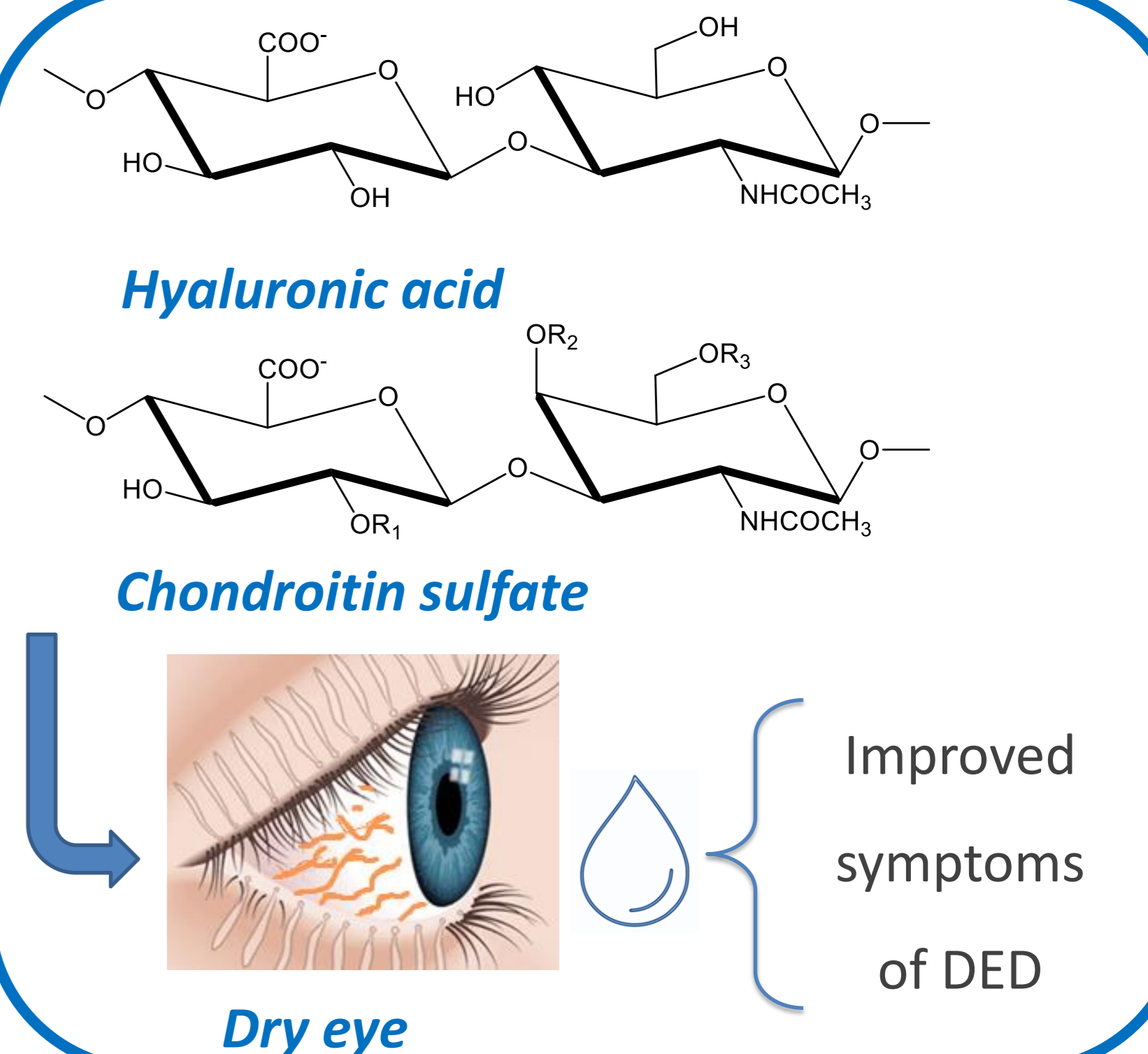


## INTRODUCTION

**Hyaluronic Acid (HA)** and **Chondroitin Sulfate (CS)** have shown improvements in the corneal epithelial barrier in patients with **Dry Eye Disease (DED)**<sup>2</sup>. They are used in pharmaceutical applications due to their biocompatibility, viscoelasticity, lubricity and immunostimulatory effects. In this study, they are extracted from **marine wastes** using **natural, green and novel solvent systems** to replace time-consuming and expensive conventional methods and maintaining the quality and purity of the isolated HA and CS. The solvent systems are based on **natural terpenes** with therapeutic effects. HA, CS and the terpene-based solvent systems will be evaluated for DED treatment<sup>3,4</sup>.

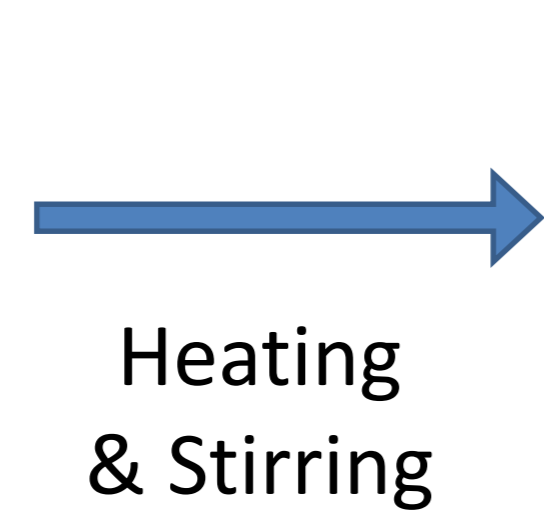
## AIM



## METHODS

### 1. Solvent systems preparation

Combinations of Menthol, Camphor, Thymol & Borneol

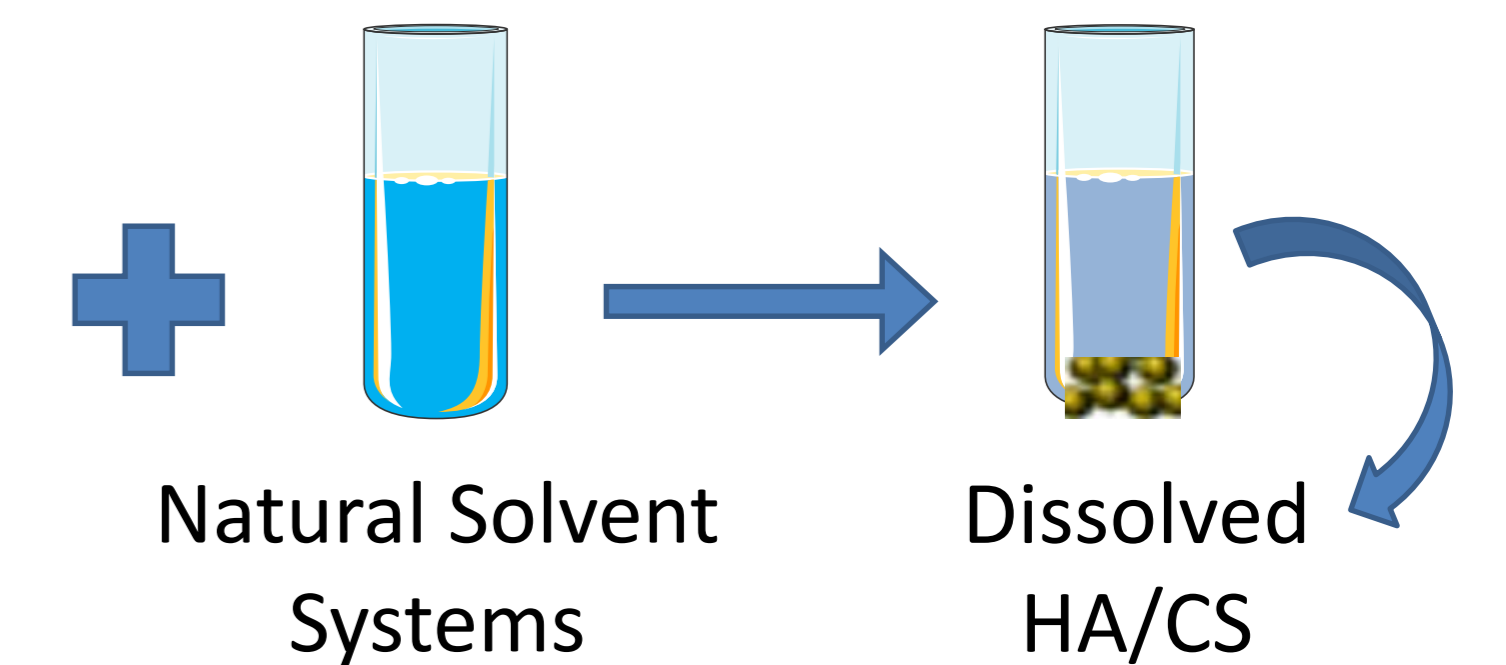


Natural Solvent Systems

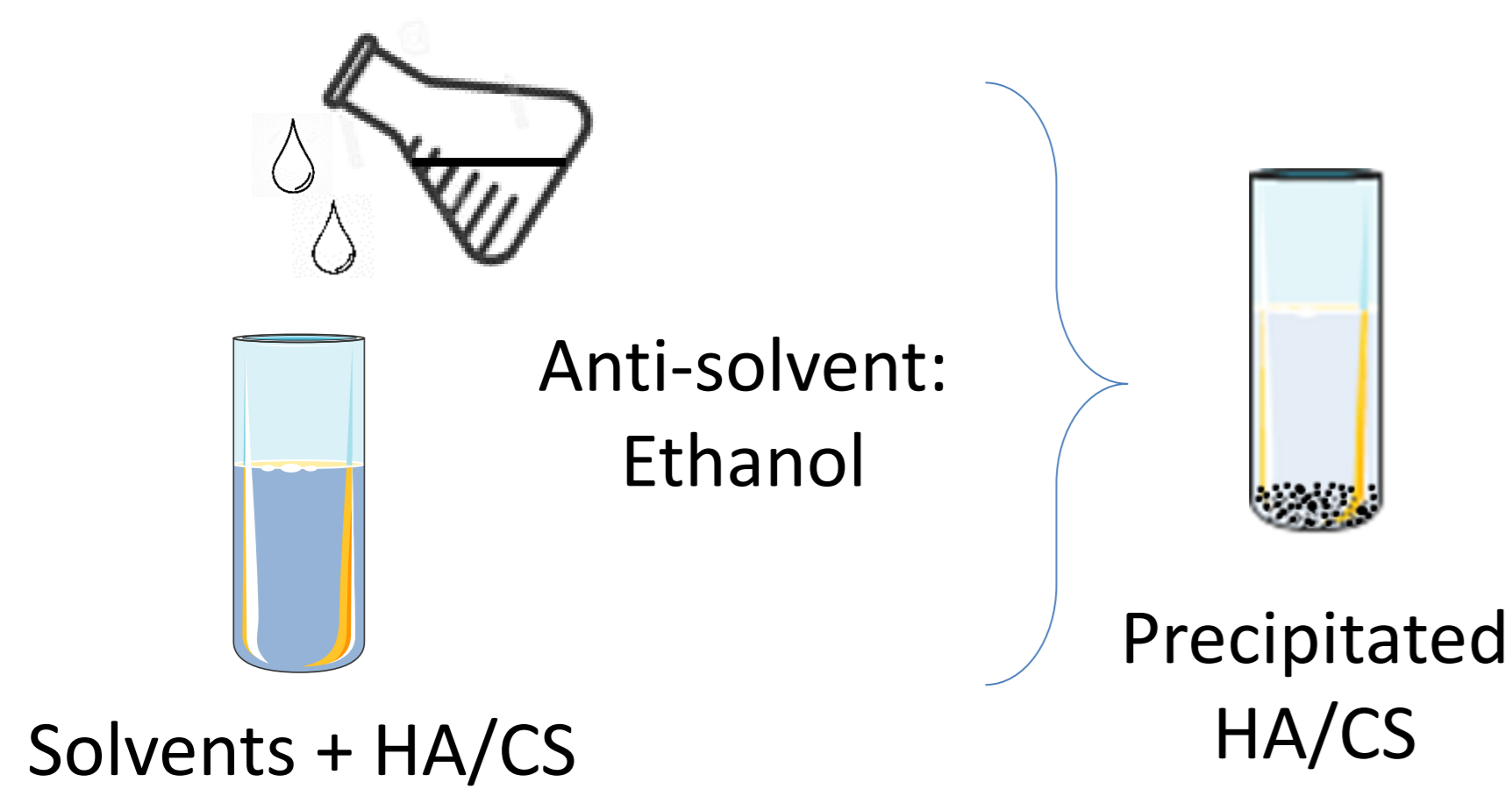
### 2. Extraction from marine by-products



Marine by-products

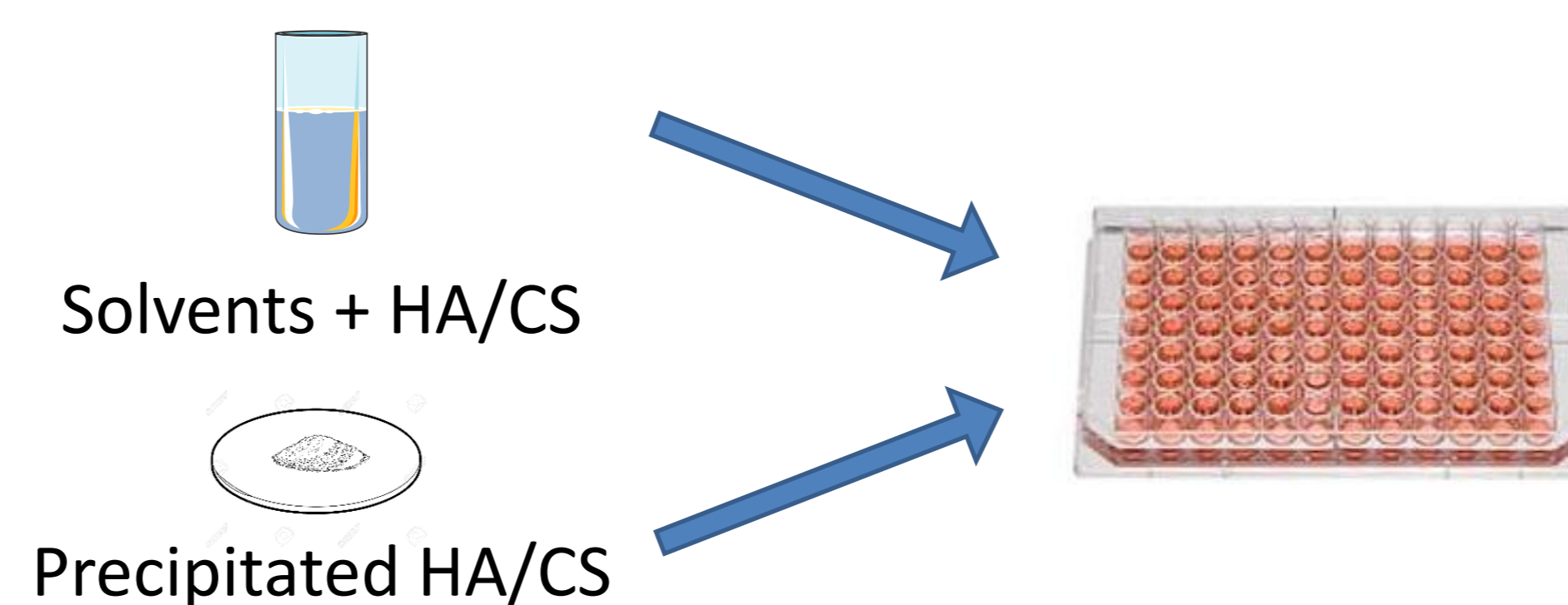


### 3. HA and CS precipitation



### 4. Bio-assays

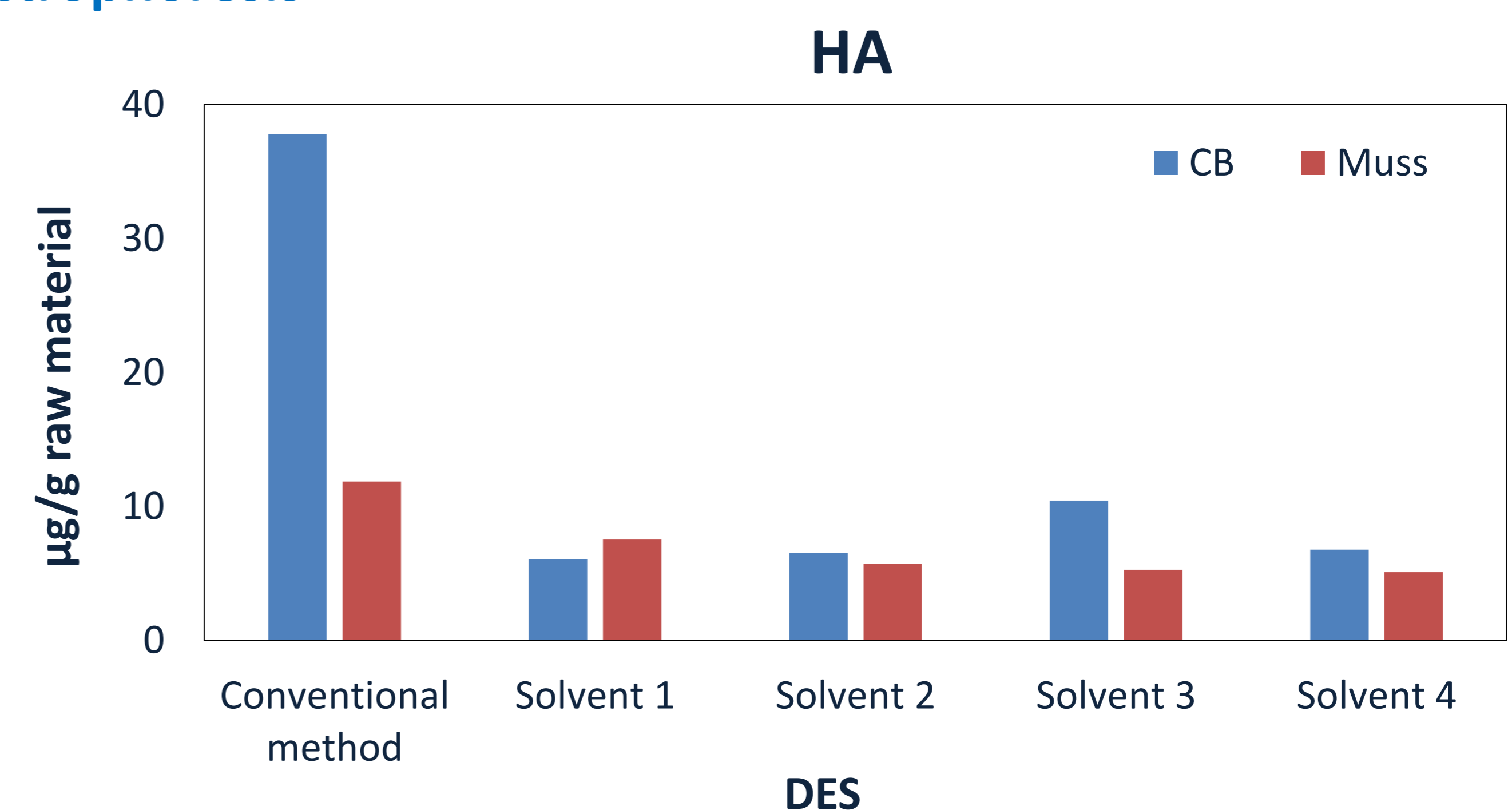
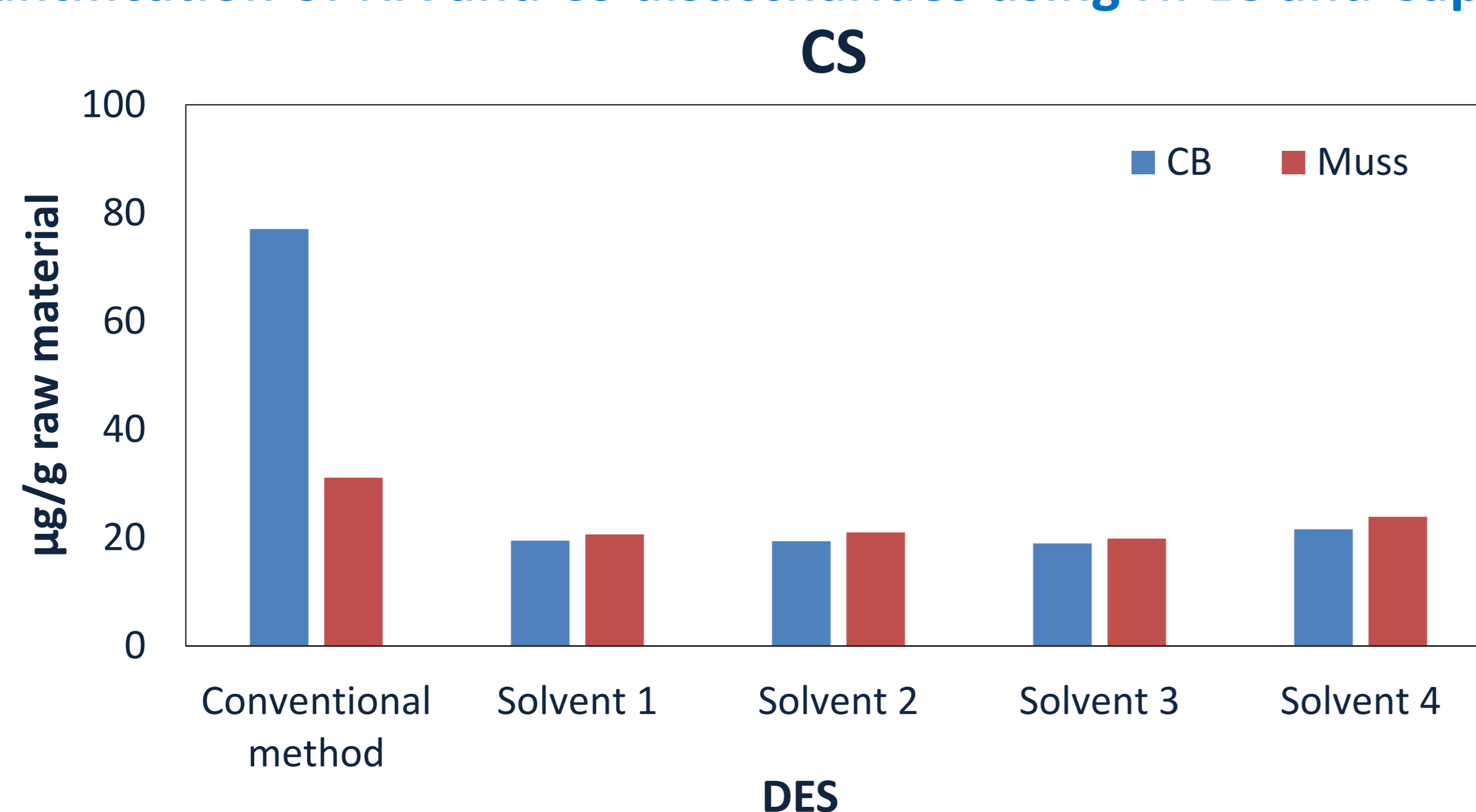
*In vitro* studies on conjunctival, corneal and epithelial human cell lines of the therapeutic mixture of the solvents + HA/CS & the isolated HA/CS



- Cytotoxicity
- Anti-oxidant activity
- Anti-inflammatory effect
- Muco-adhesion
- Cellular uptake

## RESULTS

### Quantification of HA and CS disaccharides using HPLC and Capillary Electrophoresis



## CONCLUSION

HA and CS are efficiently extracted from marine sources using a novel, green and low-cost technique by their dissolution in terpene-based solvent systems. HA and CS as well as the terpene solvent systems are currently being tested to assess their safety and applicability in the dry eye disease treatment.

## REFERENCES

- [1] Craig, Jennifer P., et al. "TFOS DEWS II report executive summary." *The Ocular Surface* 15.4 (2017): 802-812.
- [2] Limberg, Michael B., et al. "Topical application of hyaluronic acid and chondroitin sulfate in the treatment of dry eyes." *American journal of ophthalmology* 103.2 (1987): 194-197.
- [3] Arita, Reiko, et al. "Effects of a warm compress containing menthol on the tear film in healthy subjects and dry eye patients." *Scientific reports* 7 (2017): 45848.
- [4] Fondi, Klemens, et al. "Effect of Hyaluronic Acid/Trehalose in Two Different Formulations on Signs and Symptoms in Patients with Moderate to Severe Dry Eye Disease." *Journal of ophthalmology* 2018 (2018).