FLOW FIELD-FLOW FRACTIONATION FOR AN ACCURATE CHARACTERIZATION OF POLYSACCHARIDES

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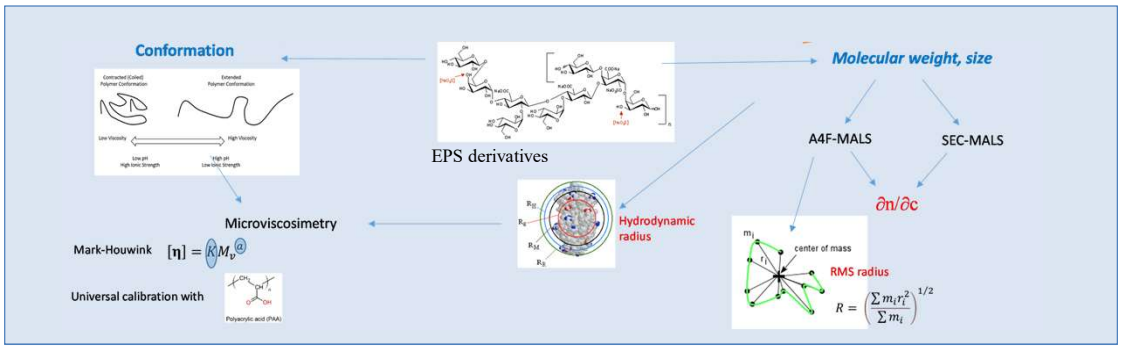
CONTEXT

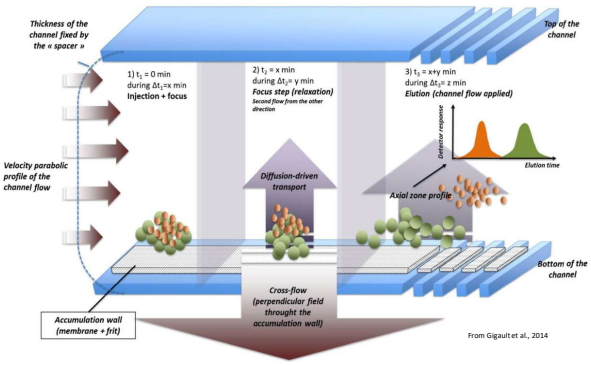
Marine sulfated polysaccharides offer a great potential for human health applications as drug for tissue regeneration or cancer therapy. GY785 EPS is an exopolysaccharide produced by the deep-sea hydrothermal bacterium, *Alteromonas infernus*. This EPS presents original structural features that can be modified to design efficient bioactive compounds and provide an alternative to the use of Glycosaminoglycans (GAGs), like heparin. This work was conducted to evaluate if low molecular weight EPS derivatives namely EPS DR and EPS DRS could be used in nuclear medicine. Chemistry, morphology, and polydispersity of low sulphated and highly sulfated derivatives have to be assessed, especially the molecular masses to perform the coupling with theranostic radionuclides.

METHODS & RESULTS

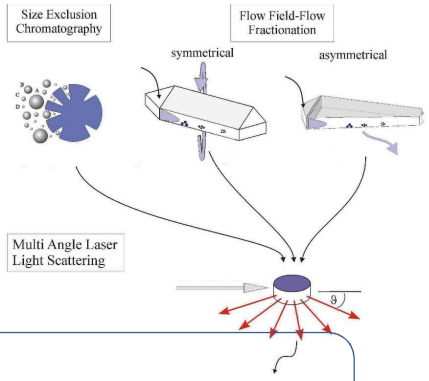
Asymetrical Flow-Field Flow Fractionation (AF4) and Size exclusion chromatography (SEC) coupled online to a multi-angle light scattering detector (MALS) and a refractometric detector are used to characterize EPS derivatives and to obtain molar masses and conformation (Ip,R_G, R_H).



AF4-MALS



In A4F, the separation is achieved in an asymmetrical flat channel. Separation depends on the Brownian diffusion coefficient of the molecule.

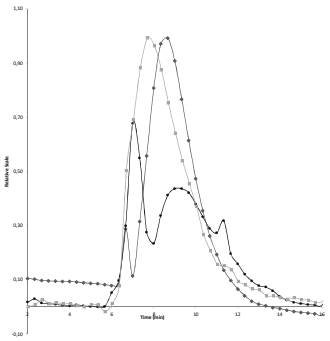


Basic Light Scattering Equation

$$\frac{K^*c}{R_\theta} = \frac{1}{M_w P_\theta} + 2A_2c \quad \text{With } K^* = \frac{4\pi^2 n_0^2 (\partial n/\partial c)^2}{\lambda_0^4 N_A}$$

$(\partial n/\partial c)$ of EPS is necessary to obtain accurate M_w from MALS

Sample	∂n/∂c (mL/g)	SO_4^{2-} %
EPS-DR	0.125 (0.001)	10
EPS-DRS	0.110 (0.001)	45



Fractogram of EPS-DRS (squares: UV-VIS @ λ = 254 nm; circles: dRI; diamonds: MALS @ 90°). [EPS-DRS] = 2 mg mL^{-1}; injection volume = 20 µL; eluent = MilliQ water with 0.1% in volume of Tween 20; membrane = PES 5 kDa; canal length = 291 mm; spacer = 350 µm; cross flow = from 2 to 0 mL min^{-1} in 17 min; injection flow = 0.20 mL min^{-1}; focalization flow = 2 mL min^{-1}; detector flow = 1 mL min^{-1}.

SEC-MALS

Elution of EPS derivatives is done on a PL aquagel-OH mixed 8 µm column (Agilent). Injected volume = 100 µL at 2 mg. mg $^{-1}$ Eluent = 0,1 M ammonium ; flow = 1 mL min^{-1} d*n*/d*c* value of 0.145 mL/g (generic for polysaccharide).

co-exclusion and polyelectrolyte effects of polysaccharide can explain significant underestimates for M_w.

	M_w (kDa)	M_n (kDa)	Ip = M_w/M_n	R_g (nm)
EPS-DR	26.6 (3.2)	18.8 (2.8)	1.41 (0.38)	44.6 (0.1)
EPS-DRS	57.0 (8.0)	39.1 (4.1)	1.46 (0.36)	58.8 (0.1)

	M_w (kDa)
EPS-DR	17,9
EPS-DRS	25,1

conformation

	Low sulphated EPS DR	Highly sulphated EPS DRS
R_g (nm)	44.6	58.8
R_h (nm)	61.2	52.7
R_G/R_H	0.7	1.1

CONCLUSION

More accurate Mw values of EPS were measured using AF4-MALS than using SEC-MALS due to

i) exact dn/dc value use
ii) no shearing degradation
iii) understanding of the polyelectrolyte effect on the separative technique

Robust rheological methods have been set for characterizations of these complex macromolecules, usable for the study of the EPS-metals interactions

1] Senni and al.,Marine polysaccharides: A source of bioactive molecules for cell therapy and tissue engineering,2011, Marine drugs.[2]Heyman and al. Anti-Metastatic Properties of a Marine Bacterial Exopolysaccharide-Based Derivative Designed to Mimic Glycosaminoglycans. Molecules,2016.[3]Mazza and al: Marine exopolysaccharide complexed with scandium aimed as theranostic agents, 2021, Molecules .