

Rheology of Hagfish Mucins

Suzanna Melotti †, Gavin Braithwaite †,
Douglas Fudge ‡, John Gosline ‡

† Cambridge Polymer Group,
52R Roland Street
Boston, MA 02139
USA

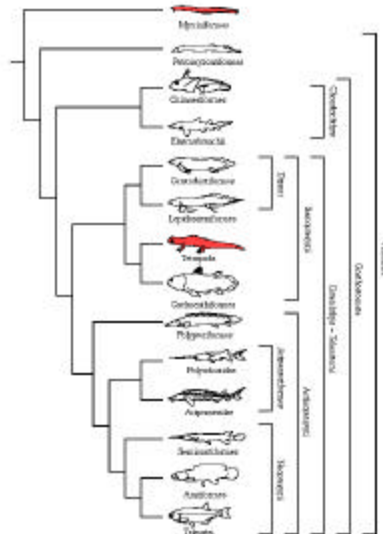
‡ Department of Zoology
University of British Columbia
6270 University Blvd
Vancouver, British Columbia V6T 1Z4
Canada



**Cambridge
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Testing, Consultation, and Instrumentation for Polymeric Materials

Hagfish: Introduction 1



Eel-shaped jawless fish

- Approximately 20 species worldwide
- Probably closely related to common ancestor of all craniates (all animals with skulls)
- Fossil hagfish from 380 million years ago
- Cartilaginous
- Active predators but also renowned as scavengers.
- Exploited for “eel skin”

University of Minnesota, College of Fisheries and Wildlife

Hagfish: Introduction 2

- Jawless
 - Rasp on tongue
- Weak eyes but strong sense of smell and touch
- Highly mobile body
 - Ties body into knots to obtain leverage and to clean itself
- Generally bottom dwellers
- Almost iso-osmotic with surrounding environment
- Generator of large amounts of “slime” as defence reaction



Image: Douglas Fudge

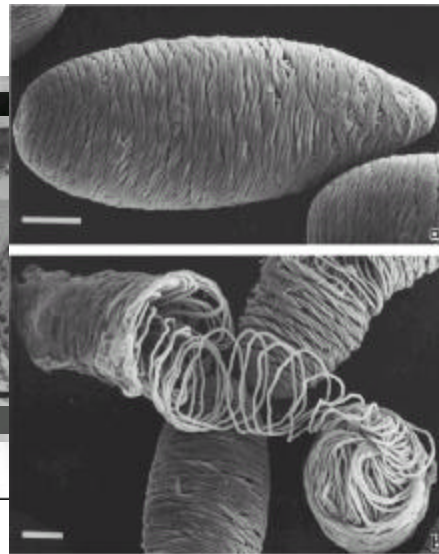


Image: BBC "Weird Nature"



Hagfish: Slime

- Slime expressed in form of thread cells and mucin vesicles in roughly equal volumes
 - 150-200 slime glands
 - Gland contains both threads and vesicles
 - Thread cells
 - Cell >150 μm long
 - Filament 1-3 μm diameter, 10-20 cm long
 - Vesicles
 - Mucous glycoproteins
 - High molecular weight (39 kDa to 10 MDa)
 - Associative
 - Thread and mucins in approximately equal concentrations (20 mg/l)
- Combination of the materials must drive the behavior of the gel



Hagfish: Mucus



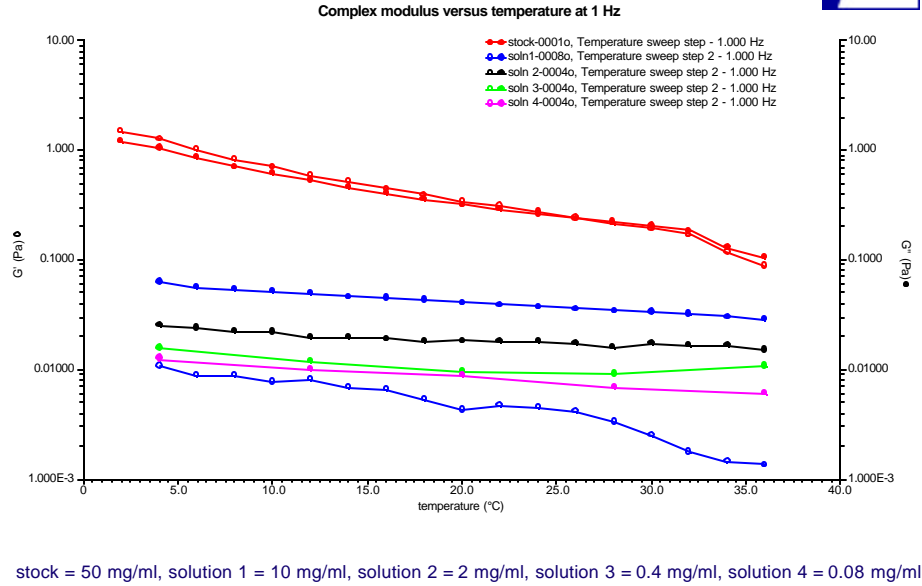
- Hagfish vesicles composed predominantly of glycoproteins (mucins)
 - Calcium ions maintain compact vesicles in gland
 - Expression results in diffusion of calcium and destruction of vesicle
 - Mucins rapidly hydrate and swell (giant granules of the slug swells approximately 600-fold in 20-30 ms)
- Mucins are polypeptides with carbohydrate chains attached usually to the serine or threonine peptides.
 - The backbone is usually predominantly threonine, proline and serine
 - Carbohydrate chains compose >70% of total mass
- Mucins ubiquitous in nature as protective gels (respiratory, intestinal, reproductive) or for transport (snails) etc.
 - Known to associate and respond to electrolyte concentrations
 - Sol-gel transitions seen at approximately 10-15 mg/ml (Bromberg and Barr (2000))
 - Physiological ~20 mg/ml
 - Often strong temperature dependence

Hagfish: Materials



- Pacific Hagfish (*Eptatretus Stoutii*) obtained from Bamfield, BC
 - Collected using traps at 100 m in Barkley sound
 - Stored in aquarium
- Slime exudate collected according to Ferry (1941) and Downing et al. (1981)
 - Slime exuded using electronic stimulation
 - Stored in high osmolarity buffer
 - (0.9M sodium citrate, 0.1M PIPES, pH 6.7)
 - Protease inhibitor added
 - Kept refrigerated
 - Use:
 - Filtered using 50 μ m mesh to remove thread cells
 - Centrifuged and supernatant drained before resuspending vesicles in DI water.
 - “Instant Ocean” added to mimic sea water.
 - pH ~9, Chloride 19.1 g/l, Sodium 10.6 g/l, sulfate 2.7 g/l, magnesium 1.3 g/l, calcium 0.4 g/l, potassium 0.4 g/l plus other trace elements
 - 5 concentrations 50 mg/l, 10 mg/l, 2 mg/l, 0.4 mg/l, 0.08 mg/l
- Data taken on AR1000 controlled stress cone and plate rheometer with 6 cm, 1° acrylic cone.

Hagfish: Temperature sweep

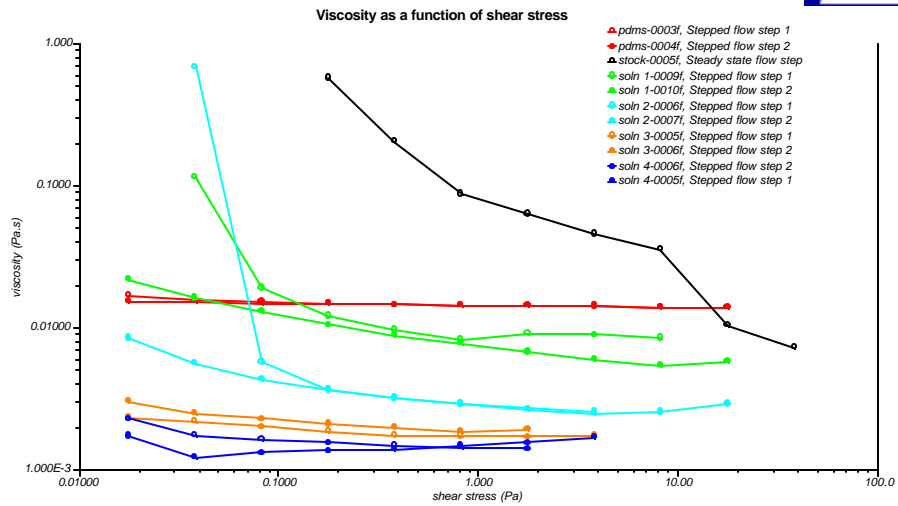


Hagfish: Materials



- Temperature sweep (oscillation)
 - No strong temperature dependence
 - Highest concentrations predominantly elastic, Lowest concentrations purely viscous
 - Cross-over suggested at between 10-50 mg/ml
 - Possible weak transition in temperature at 30 C?
 - Low viscosity results in poor instrument resolution (bottom of torque range)

Hagfish: Stress sweep and hysteresis



stock = 50 mg/ml, solution 1 = 10 mg/ml, solution 2 = 2 mg/ml, solution 3 = 0.4 mg/ml, solution 4 = 0.08 mg/ml

Hagfish: Materials

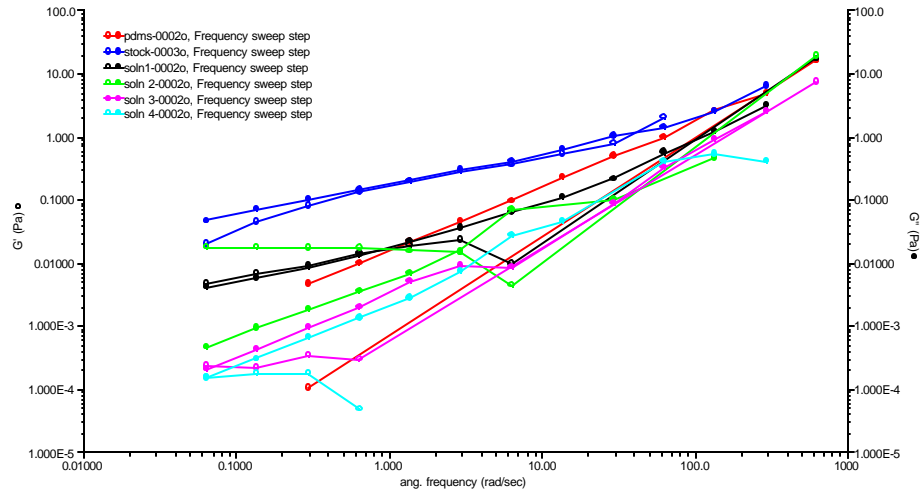


- Steady shear stress sweep
 - Sign of yield stress at higher concentrations
 - 50, 10 and 0.2 mg/ml all indicate some form of yield stress
 - Indication of hysteresis for up and down curves
 - Reformation timescale?
 - Small torques and displacements therefore poor data resolution
 - PDMS standard behaves as expected

Hagfish: Frequency sweep

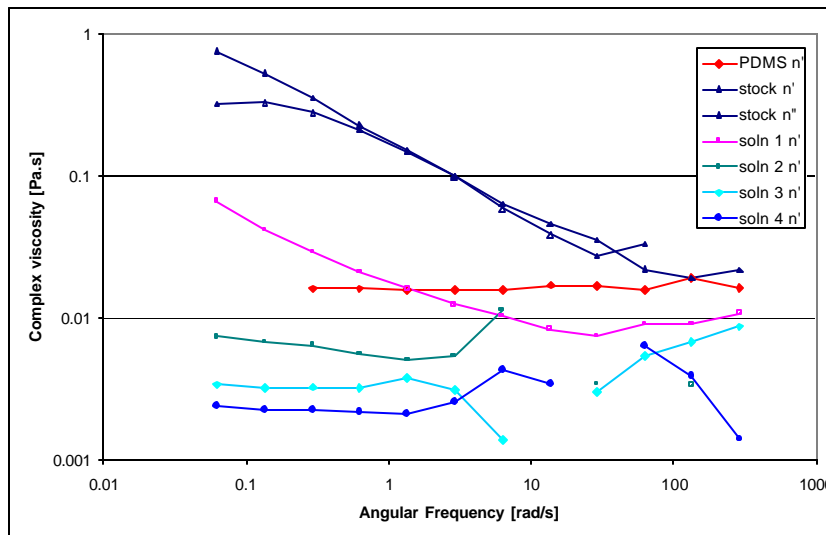


Complex modulus versus frequency at 4 C



stock = 50 g/l, solution 1 = 10 g/l, solution 2 = 2 g/l, solution 3 = 0.4 g/l, solution 4 = 0.08 g/l

Hagfish: Frequency sweep

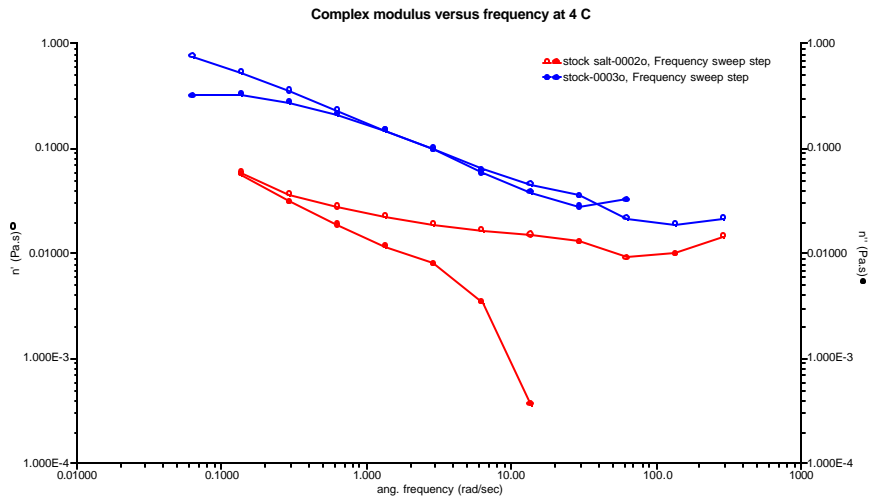


Hagfish: Materials



- Frequency sweep (1% strain)
 - Very different from previous work on mucins (Bromberg and Barr (2000))
 - Maxwell model-like at low temperatures
 - 2-3 orders of magnitude weaker

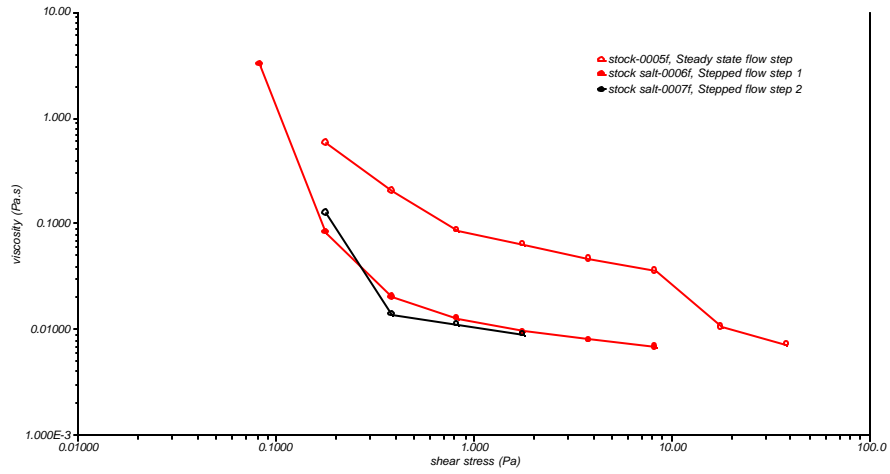
Hagfish: Effect of salt



Hagfish: Effect of salt 2



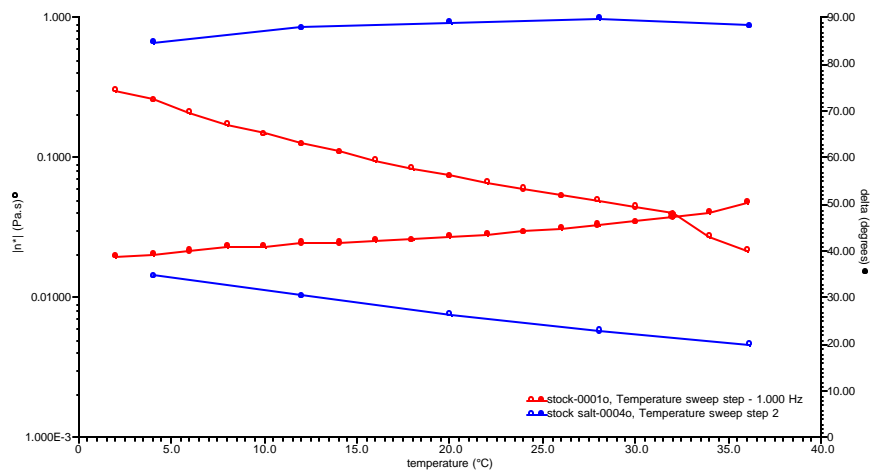
Viscosity as a function of shear stress



Hagfish: Effect of salt 3



Complex modulus versus frequency at 4 C



Hagfish: Conclusions

