Molecular and Rheological Properties of Sodium Hyaluronate and Equine Synovial Fluid

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December 15, 2004

Outline

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  - Rheological characterization theory and results
- Equine synovial fluid characterization
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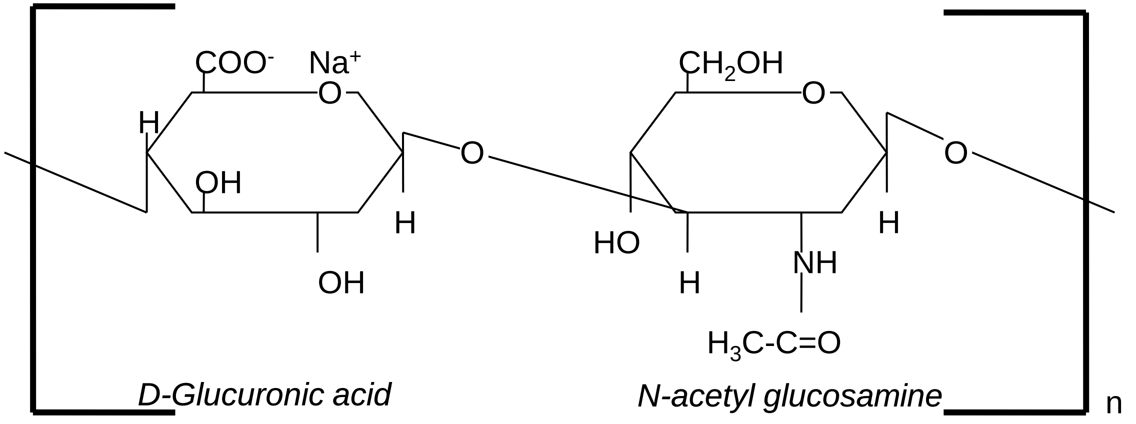
- Intra-articular study
  - Rheological Results
- Conclusions

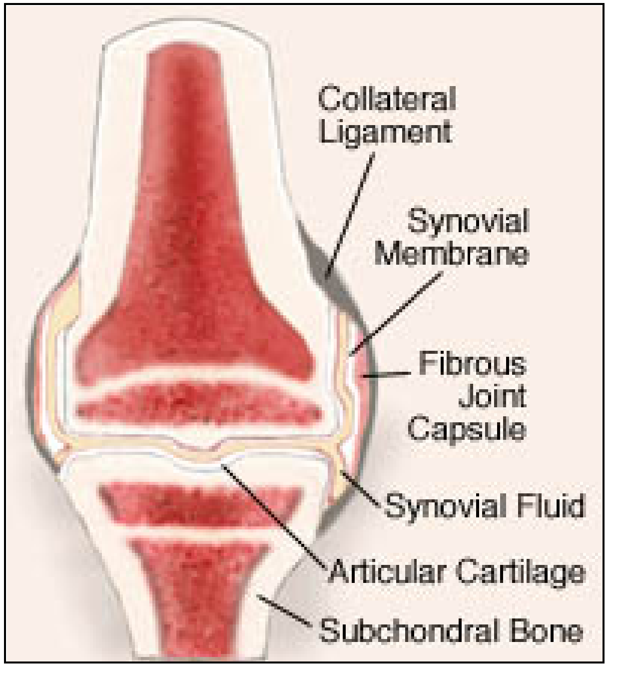
Introduction: Sodium Hyaluronate



- Sodium hyaluronate, hyaluronan, hyaluronic acid (HA)
- Biologically ubiquitous
- High molecular weight, 0.2-10 million Dalton (Da)
- Sources include fermentation, umbilical cords, chicken combs

Introduction: Role in joint lubrication

- HA is major component in synovial (joint) fluid
  - Contributes *viscous* properties for lubrication
  - Contributes *elastic* properties for shock absorption
- HA is *viscoelastic*
- Viscoelastic properties dependent on HA-HA interactions
- Interactions = f(concentration, molecular weight)



Motivation

- Correlate molecular weight and concentration of HA to viscoelastic properties

- Determine the difference between healthy and diseased synovial fluid

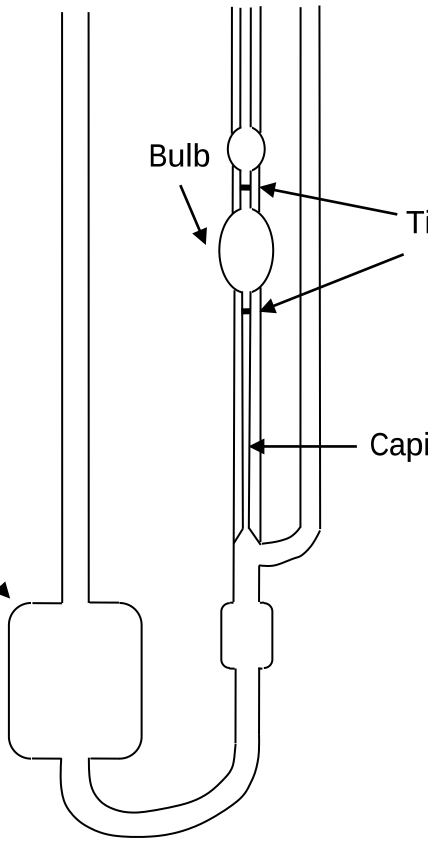
Sodium Hyaluronate Characterization

Sodium Hyaluronate Characterization

Sample	HA Source	Intended Use	HA Concentration (mg/ml)
[1]HAC3Na	Chicken Combs	Laboratory	Frozen
[1]HAC2Na	Chicken Combs	Laboratory	Frozen
[1]HAC1Na	Chicken Combs	Laboratory	Frozen
[1]HA1NaL	Umbilical Cords	Laboratory	Frozen
[1]HA2NaF	Umbilical Cords	Laboratory	Frozen
[2]Synthovial 7	Fermentation	Human Oral	3
[2]Hyalun	Fermentation	Equine Oral	5
[3]Legend	Fermentation	Equine IV	10
[4]Hyalovet	Chicken Combs	Equine IA	10
[5]Hyvisc	“natural sources”	Equine IA	11

Manufacturer
[1]Biozyme Laboratories
[2]Hyalogic
[3]Bayer Corporation
[4]Wyeth
[5]Anika Therapeutics

Molecular theory: Intrinsic viscosity



- Measures intrinsic viscosity (IV) via increasingly dilute polymer solutions

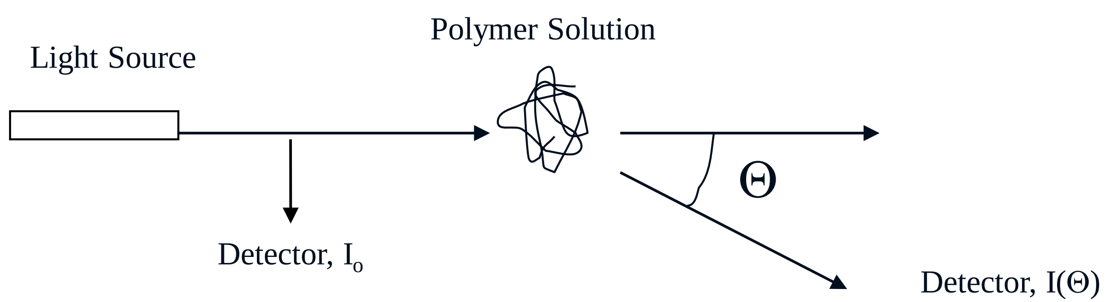
$$[\eta] = \lim_{c\to 0} \frac{\eta - \eta_s}{\eta_s c} = \lim_{c\to 0} \frac{t - t_s}{t_s c}$$

- Can calculate molecular weight given Mark-Houwink-Sakurada (MHS) parameters (K, a = constants)

$$[\eta] = K M_w{}^a$$

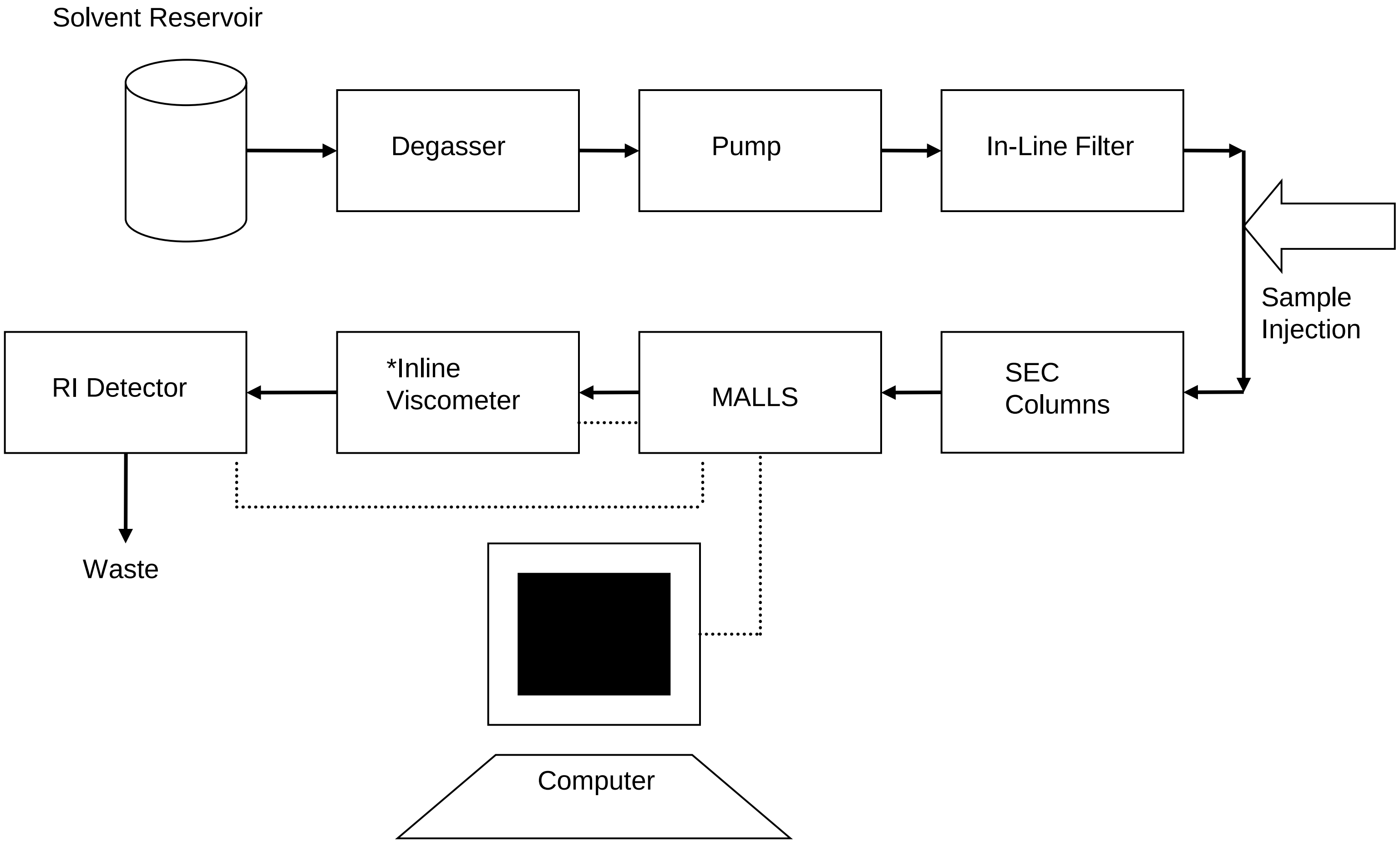
- a = 0.5 (poor solvent)
- 0.6 < a < 0.8 (good solvent)

Molecular Theory: Light Scattering



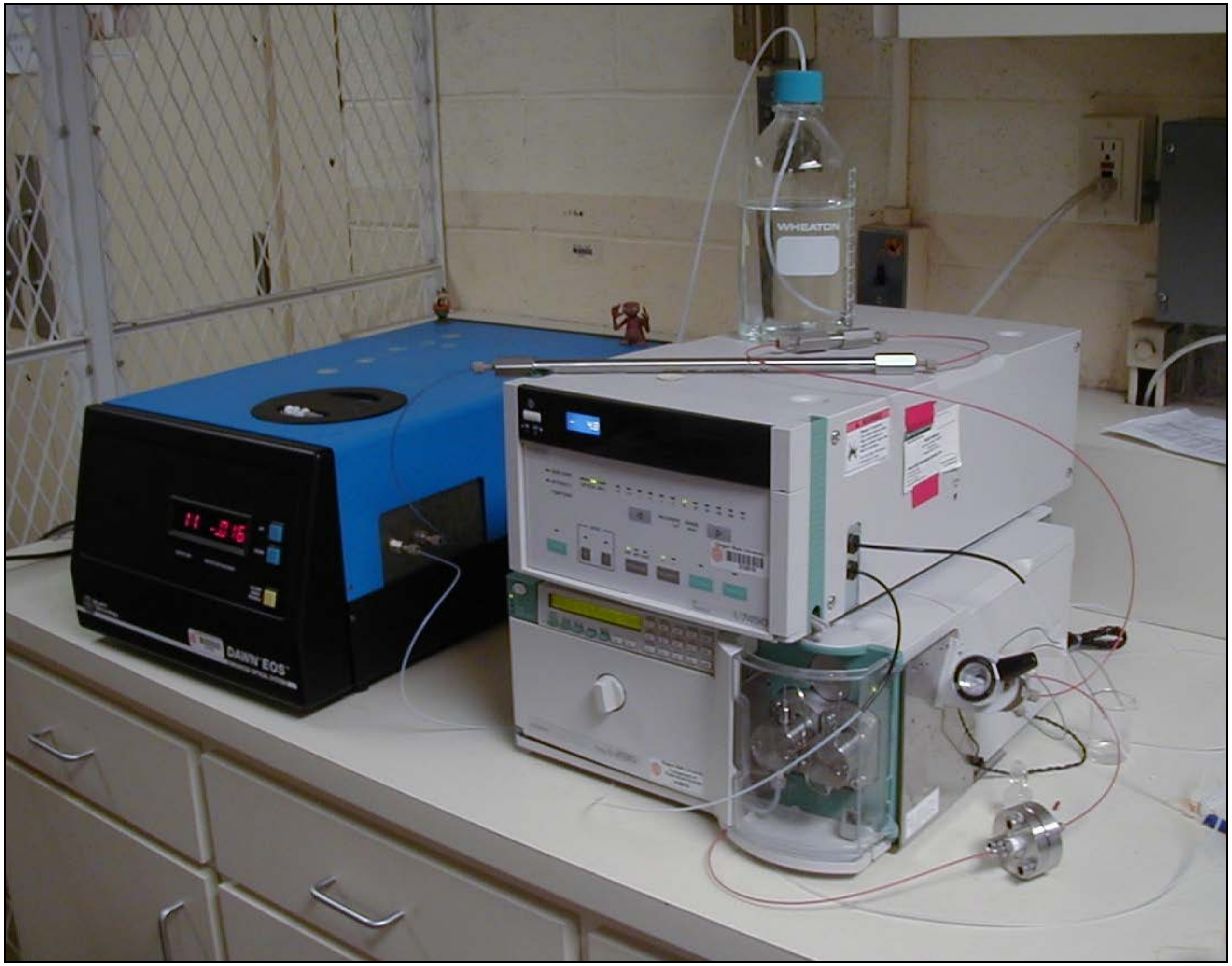
- Measure intensity of light as a function of angle and concentration
- Intensity is proportional to c, Mw
- Angular intensity based on $\langle r_g \rangle$

Size Exclusion Chromatography Multi-Angle Laser Light Scattering (SEC-MALLS)

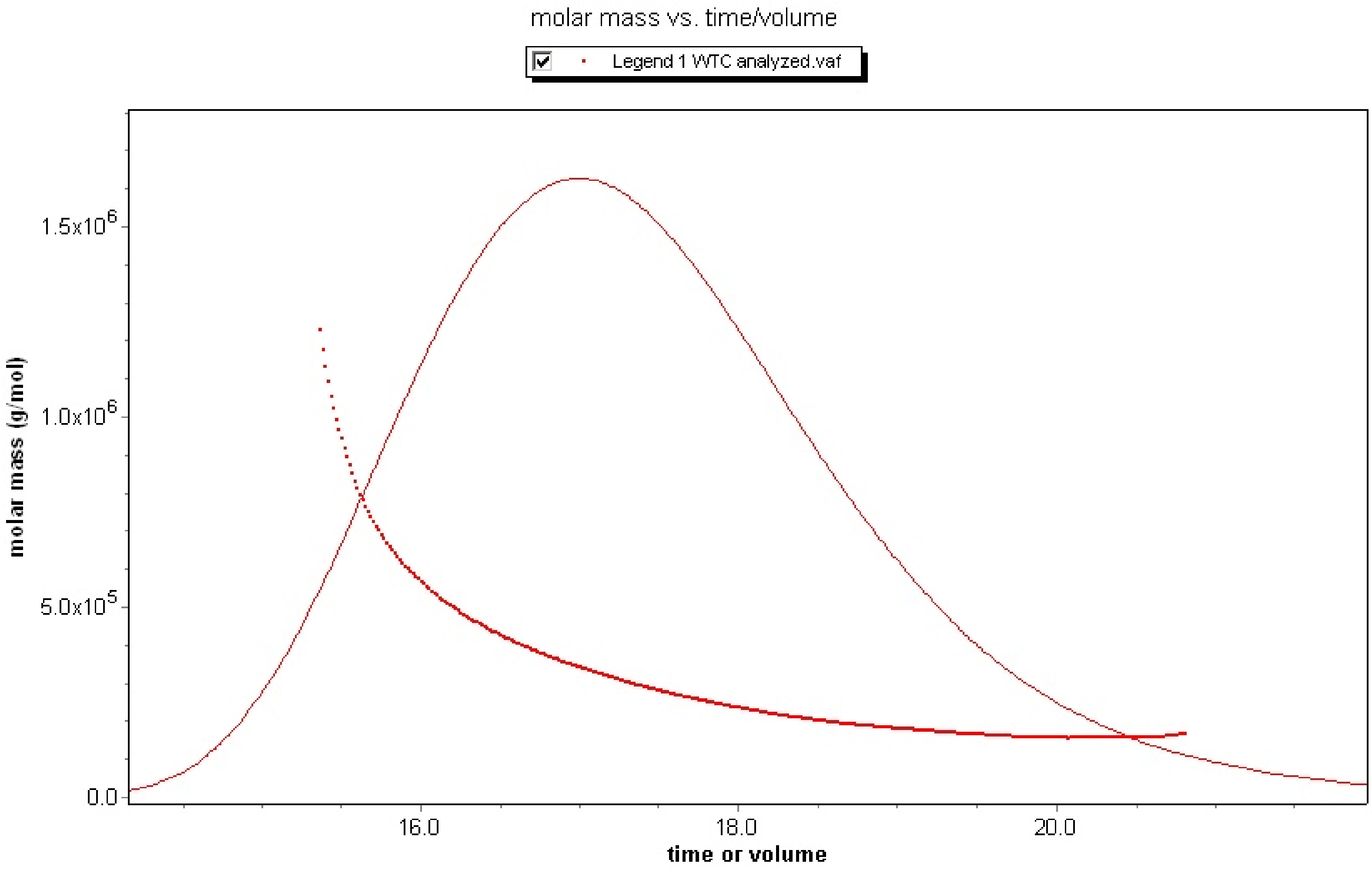


*Used at Wyatt Technology Corporation

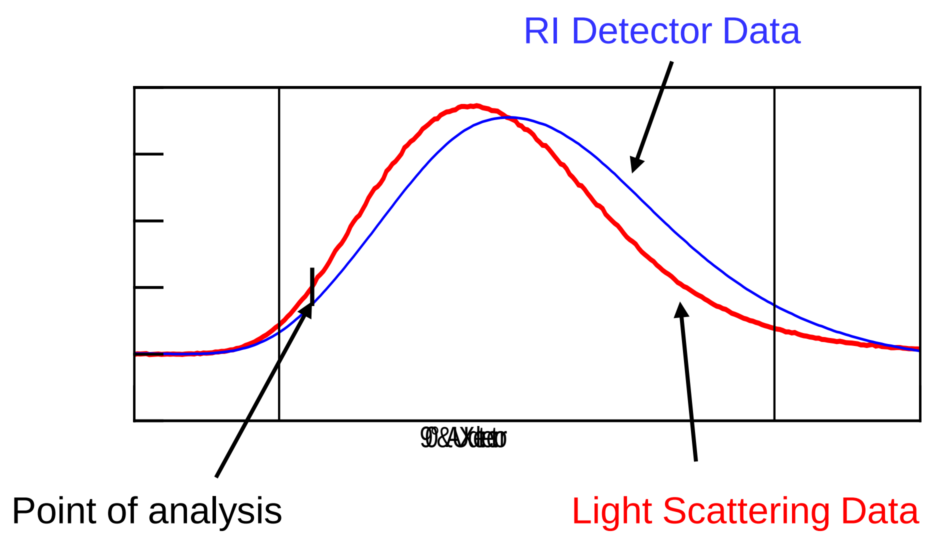
OSU SEC-MALLS System



SEC-MALLS: Example Results



SEC-MALLS: Analysis



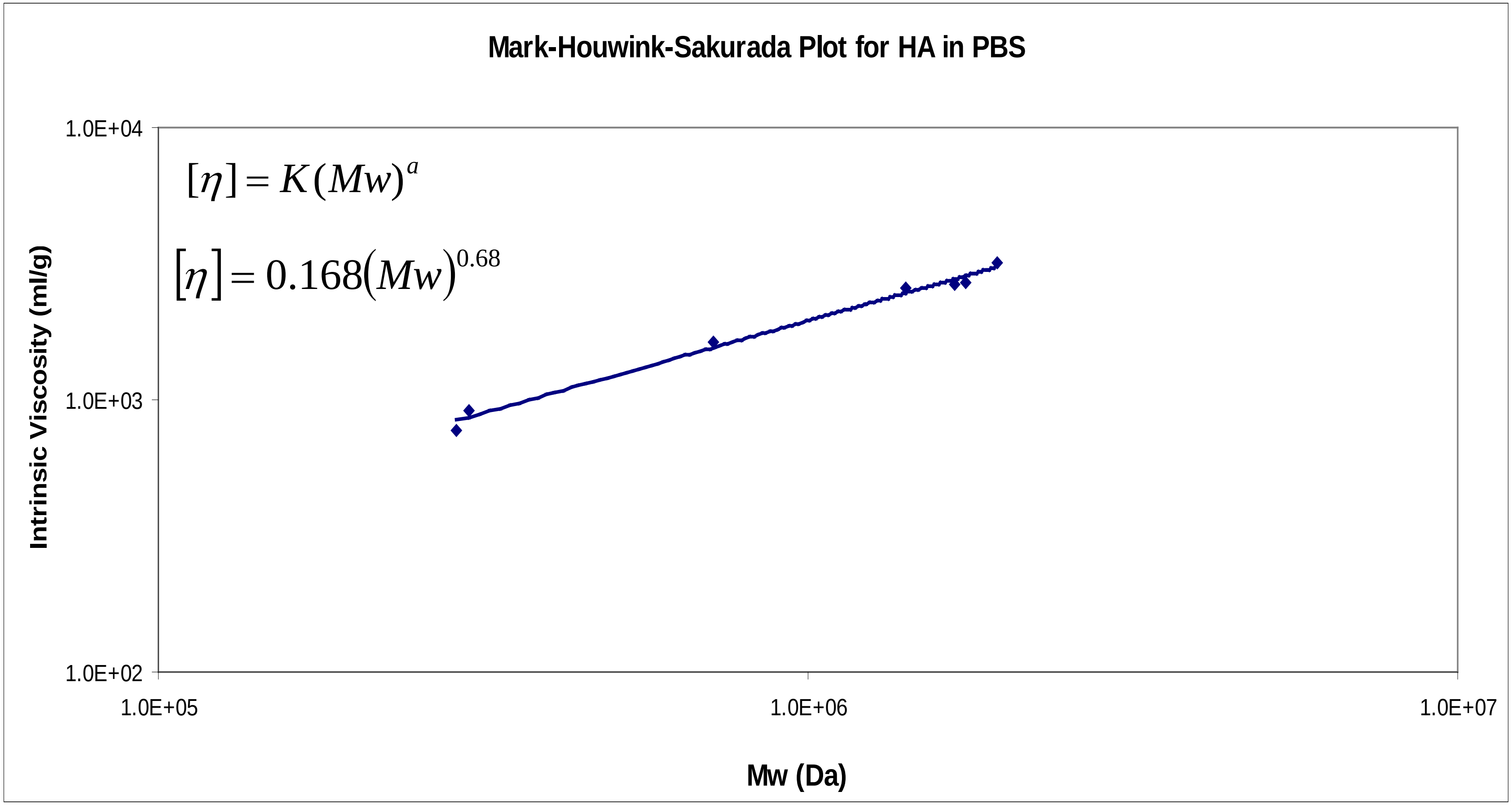
- At each point of the peak calculate
  - Mw
  - Concentration
  - Radius
  - Intrinsic viscosity

SEC-MALLS: Results

	From Wyatt Technology Corporation		
	Mw	**Mw/Mn**	**[η] (ml/g)**
HAC3Na	1.96E+06	1.06	3210.30
HAC2Na	7.16E+05	1.14	1622.00
***HAC1Na**	8.45E+05	1.18	N/A
HA1NaL	2.88E+05	1.23	776.10
HA2NaF	1.68E+06	1.05	2646.00
***Synthovial 7**	1.46E+06	1.13	N/A
Hyalun	1.41E+06	1.45	2590.80
Legend	3.00E+05	1.19	916.00
***Hyalovet**	5.01E+05	1.13	N/A
Hyvisc	1.75E+06	1.03	2690.00

[†]Mw and Mw/Mn measured at OSU

SEC-MALLS: Results



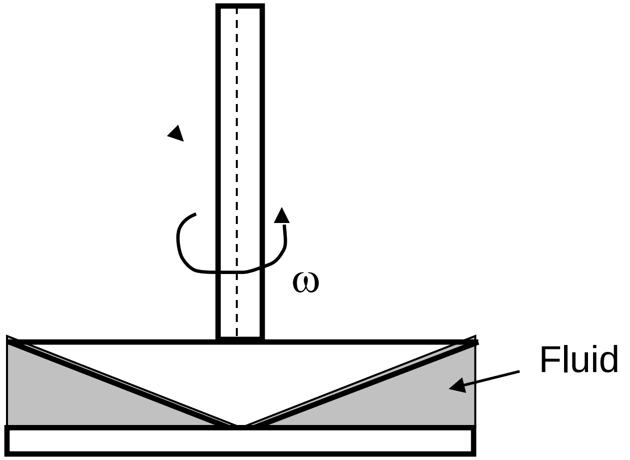
Summary of Molecular Characterization

- Light scattering used to determine molecular weight and molecular weight distribution

- Intrinsic viscosity correlated to molecular weight

$$[\eta] = 0.168(Mw)^{0.68}$$

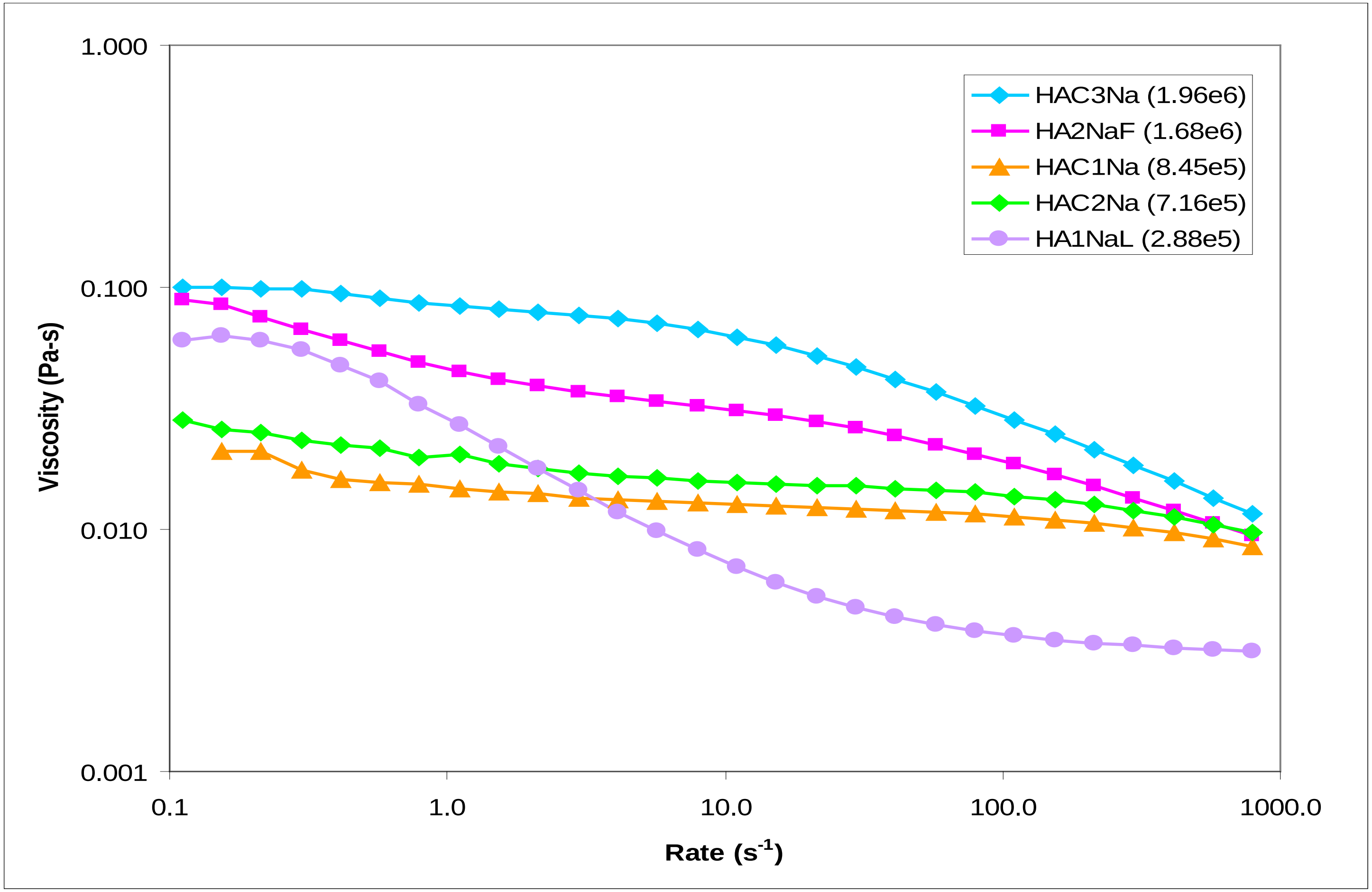
Rheological Characterization: Theory



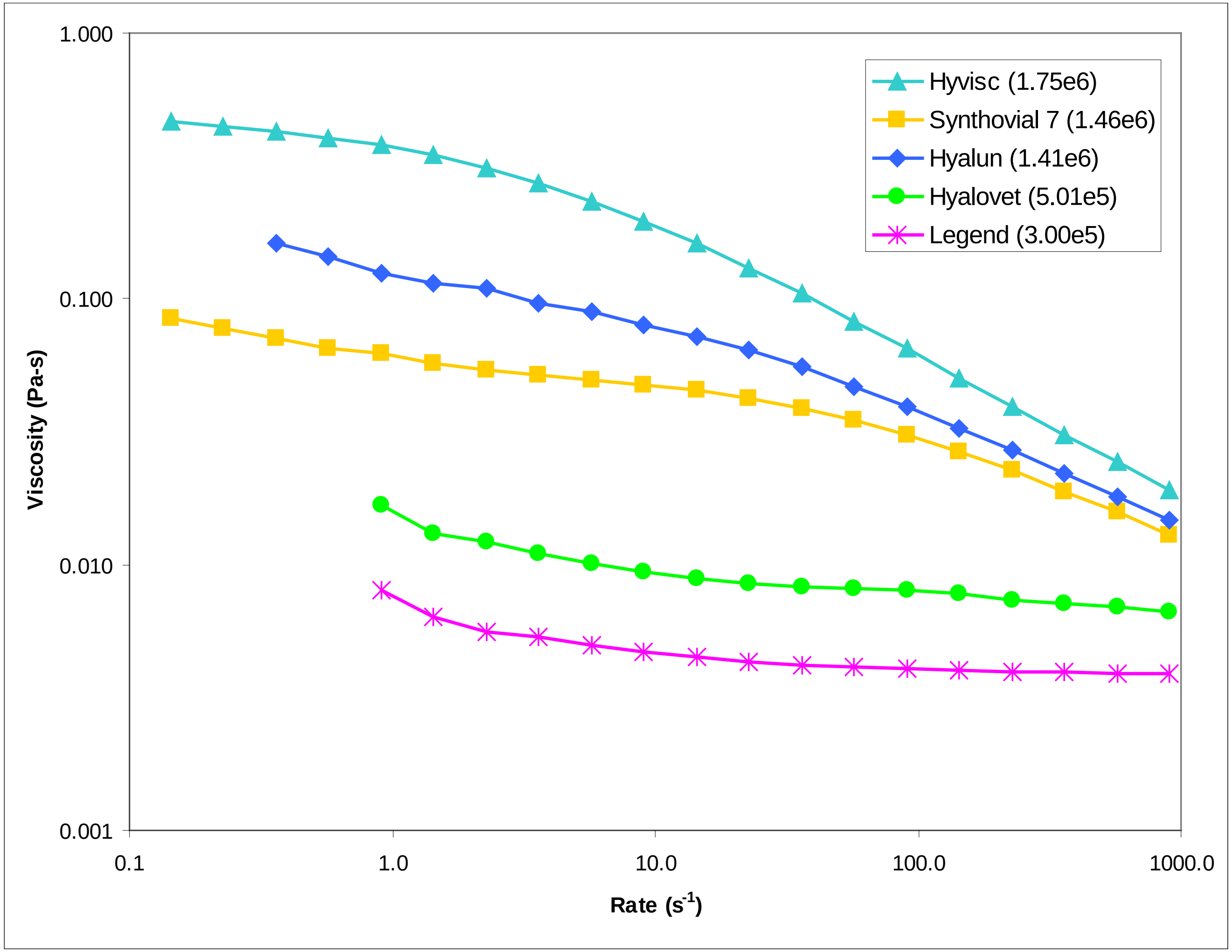
- Cone rotates at a constant speed (*shear rate*) while rheometer measures *shear stress*

$$\text{Viscosity} = \frac{\text{Shear stress}}{\text{Shear rate}} = \frac{\tau}{\dot{\gamma}}$$

Steady Shear Frozen HA Samples, 2.5 mg/ml



Steady Shear HA Supplements, 2.5 mg/ml



Sodium Hyaluronate Characterization: Conclusions

- Molecular weight and rheological properties correlate well
- HA behaves as a random coil in solution

$$[\eta] = 0.168(Mw)^{0.68}$$

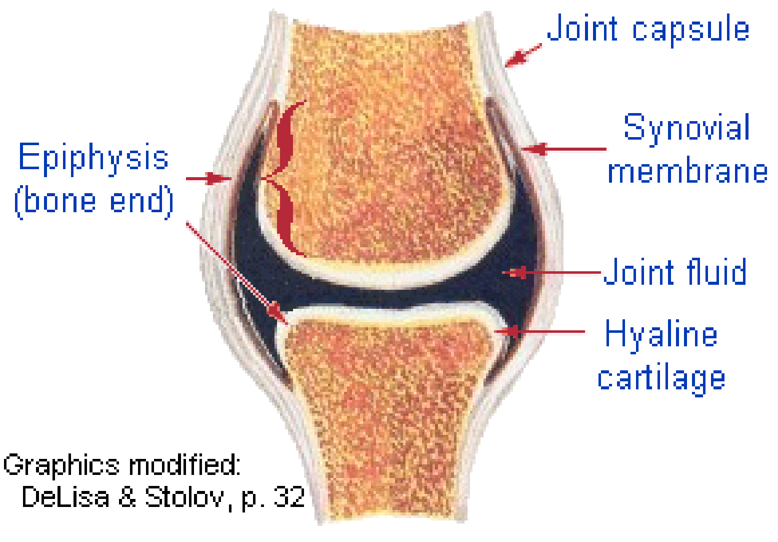
- Rheological characterization shows associations
- Molecular and rheological characterization techniques can now be applied to synovial fluid

Equine Synovial Fluid Characterization

Synovial Fluid Characterization: Introduction

- HA produced by cells in the synovial lining
- Mechanical pressure pushes HA from cartilage to joint cavity, “weeping lubrication”

Typical synovial joint



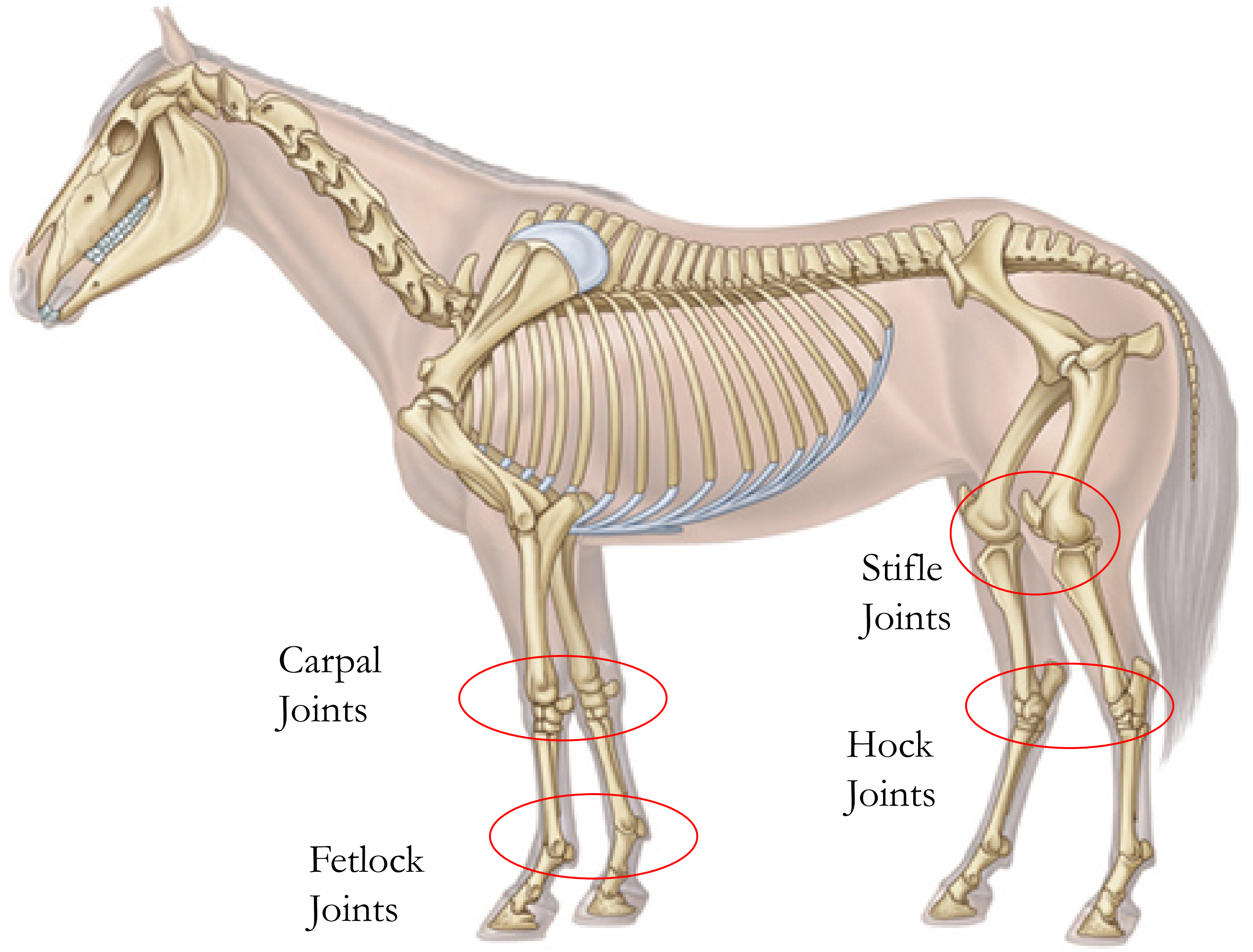
Graphics modified:
DeLisa & Stolov, p. 32

- Composed of HA, proteins, glycosaminoglycans (GAG’s) and other molecules
- HA concentration 0.1-4 mg/ml
- Mw 1×10^4 – 7×10^6 Da

Joint Disease

- HA may be broken down by radicals or enzymes with disease
  - Degenerative Joint Disease – DJD
    - Damage to the articular cartilage
    - Commonly affects heavily worked and aged horses
  - Osteochondritis Dissecans – OCD
    - Failure of the bone underlying the smooth articular cartilage to form properly
    - Commonly affects young horses

Horse Joints

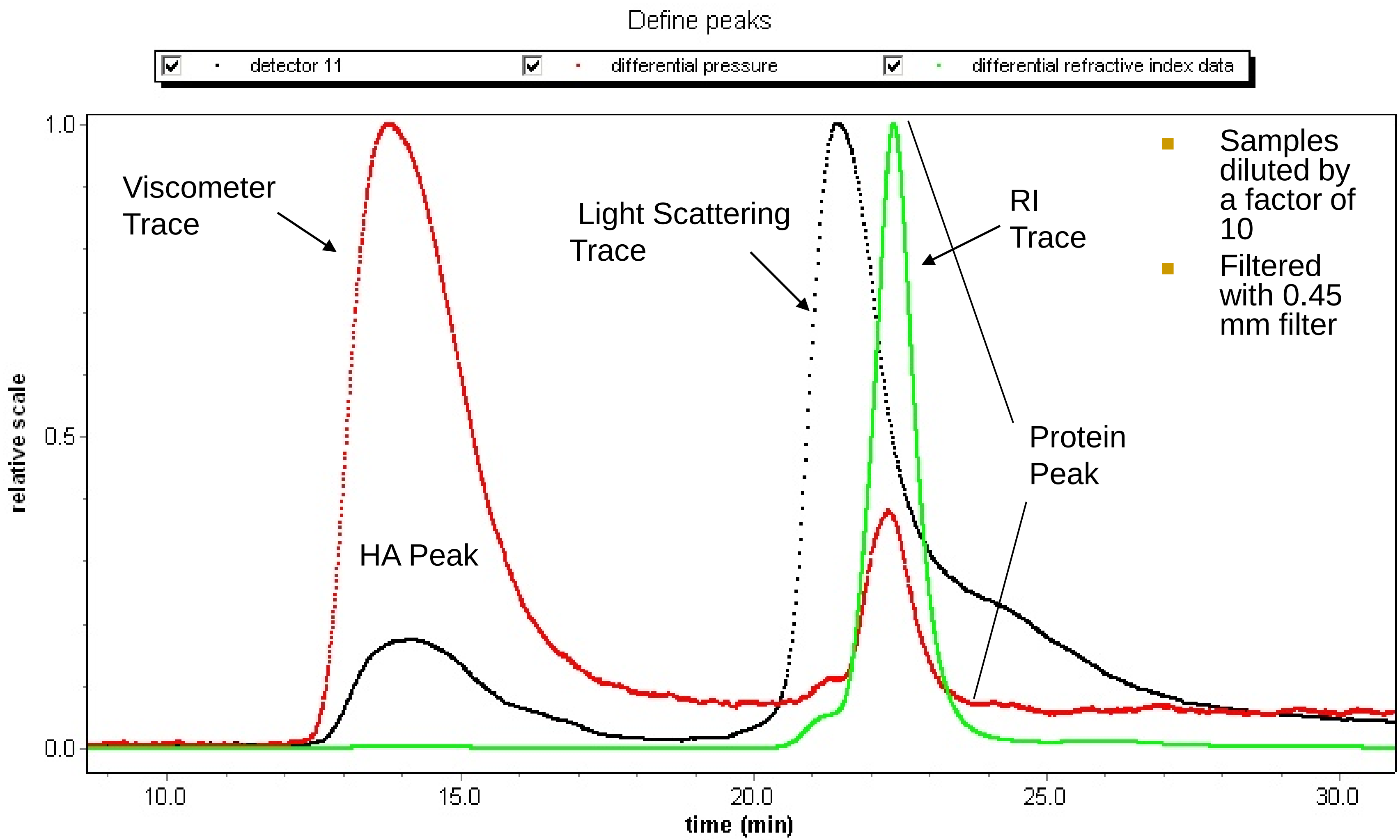


Synovial Fluid Samples

	Sample	Comments
Stifles	DH1 LS	Euthanized, left stifle
	DH2 RS	Euthanized, right stifle
	DH2 LS	Euthanized, left stifle
	DH3 RS	Euthanized, right stifle
	DH3 LS	Euthanized, left stifle
Hocks	DH3 RH	Euthanized, right hock
	DH3 LH	Euthanized, left hock
	DH1 RH	Euthanized, right hock
	VH1 LH	Live, left hock
	VH3 RH	Live, right hock
Abnormal	abDH4 RS	Euthanized, right stifle, OCD
	abDH5 LH	Euthanized, left hock, OCD
	abDH6 LH	Euthanized, left hock, lame
	abDH7 RS	Euthanized, right stifle, lame
	abDH8 RH	Euthanized, right hock, OCD

- Molecular characterization
- Rheological characterization

SEC-MALLS: abDH4 RS



- Samples diluted by a factor of 10
- Filtered with 0.45 mm filter

SEC-MALLS: Results

	Sample	Mw	*Mw St Dev*	Mw/Mn	*Mw/Mn St Dev*	c (mg/ml)	*c St Dev*
St	DH1 LS	2.64E+06	*12.7%*	1.11	*6.88%*	**0.84**	*8.1%*
	DH2 RS	5.46E+06	*36.2%*	1.01	*0.57%*	0.48	*7.2%*
	DH2 LS	3.31E+06	*13.8%*	1.01	*1.14%*	0.48	*3.9%*
	DH3 RS	4.81E+06	*18.6%*	1.14	*2.32%*	0.34	*14.5%*
	DH3 LS	2.64E+06	*15.2%*	1.06	*7.12%*	0.17	*37.1%*
Ho	DH3 RH	2.73E+06	*39.8%*	1.33	*14.68%*	0.15	*25.6%*
	DH3 LH	**6.54E+06**	*16.3%*	1.16	*14.43%*	0.13	*28.0%*
	DH1 RH	3.00E+06	*17.2%*	1.02	*1.27%*	0.13	*13.0%*
	VH1 LH	4.57E+06	*23.2%*	1.14	*2.63%*	0.31	*12.9%*
	VH3 RH	3.06E+06	*37.6%*	1.03	*0.97%*	0.22	*18.2%*
Ab	abDH4 RS	**1.56E+06**	*38.8%*	1.66	*8.08%*	0.18	*6.7%*
	abDH5 LH	2.20E+06	*16.9%*	1.27	*8.96%*	0.27	*16.7%*
	abDH6 LH	1.88E+06	*31.0%*	1.27	*7.06%*	0.18	*22.2%*
	abDH7 RS	4.93E+06	*13.2%*	1.01	*0.99%*	0.32	*9.7%*
	abDH8 RH	5.14E+06	*23.3%*	1.26	*21.72%*	**0.11**	*76.7%*

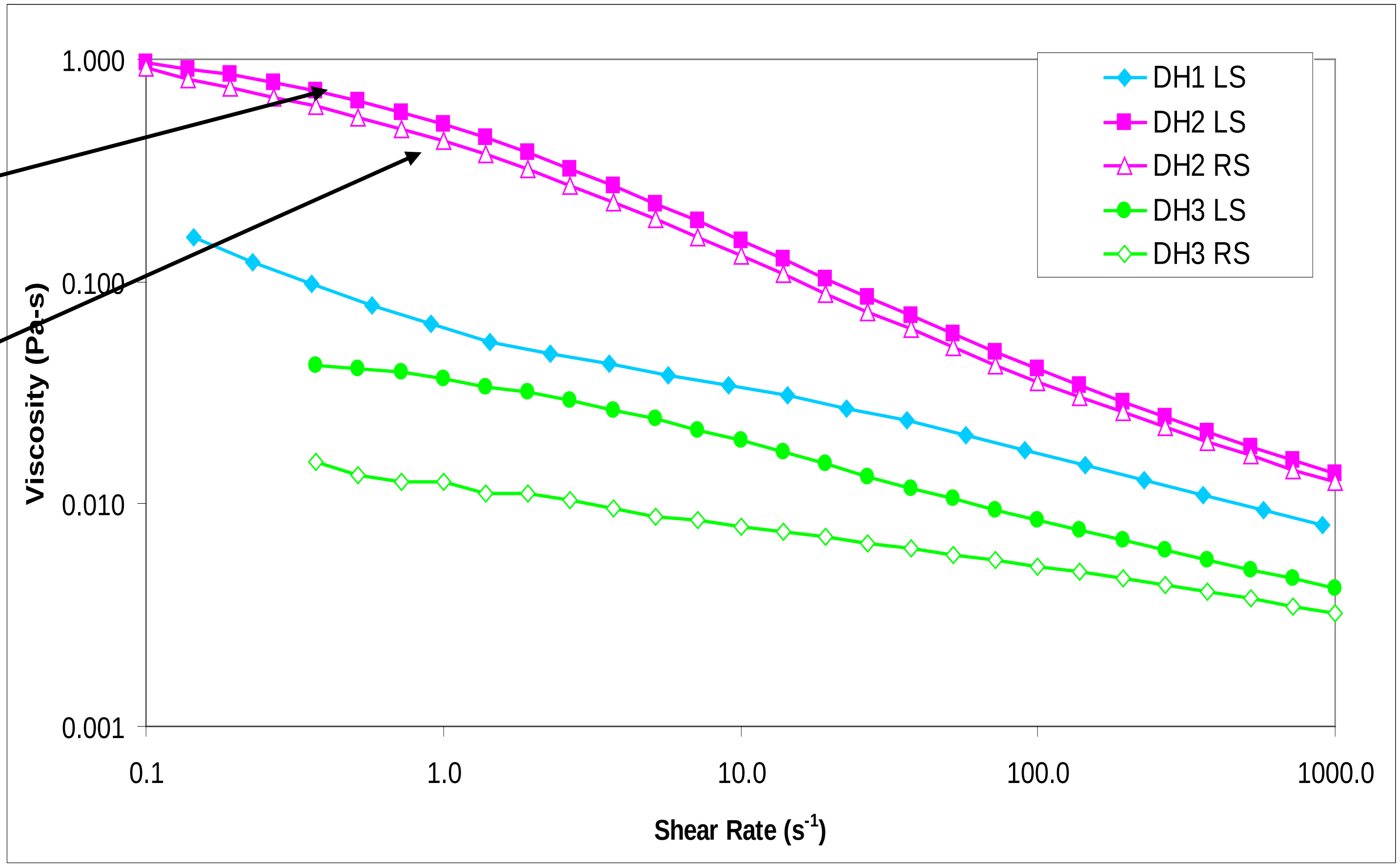
Rheology
Steady Shear: Stifles

Mw = 3.31x10⁶

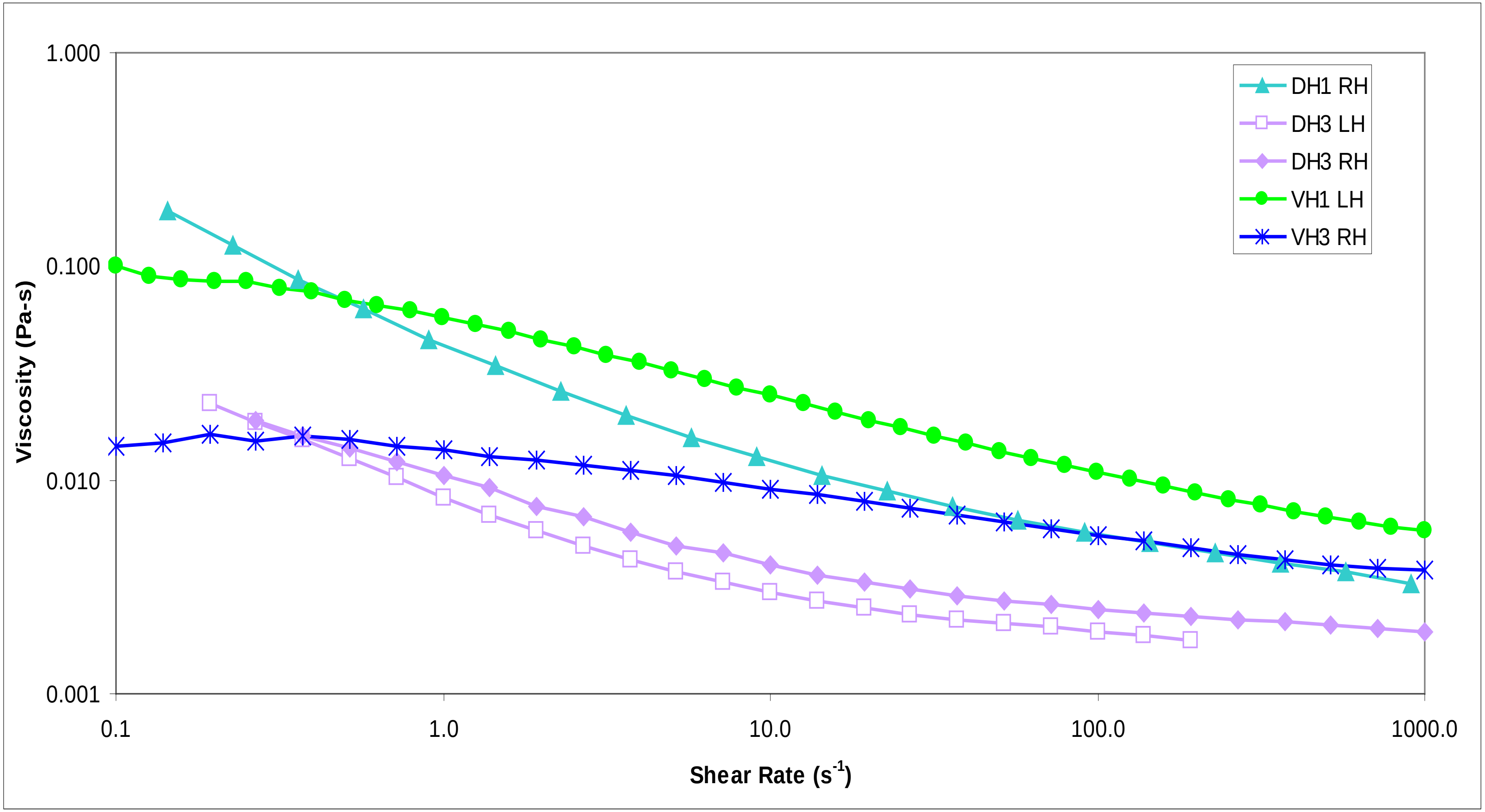
c = 0.48

Mw = 5.46x10⁶

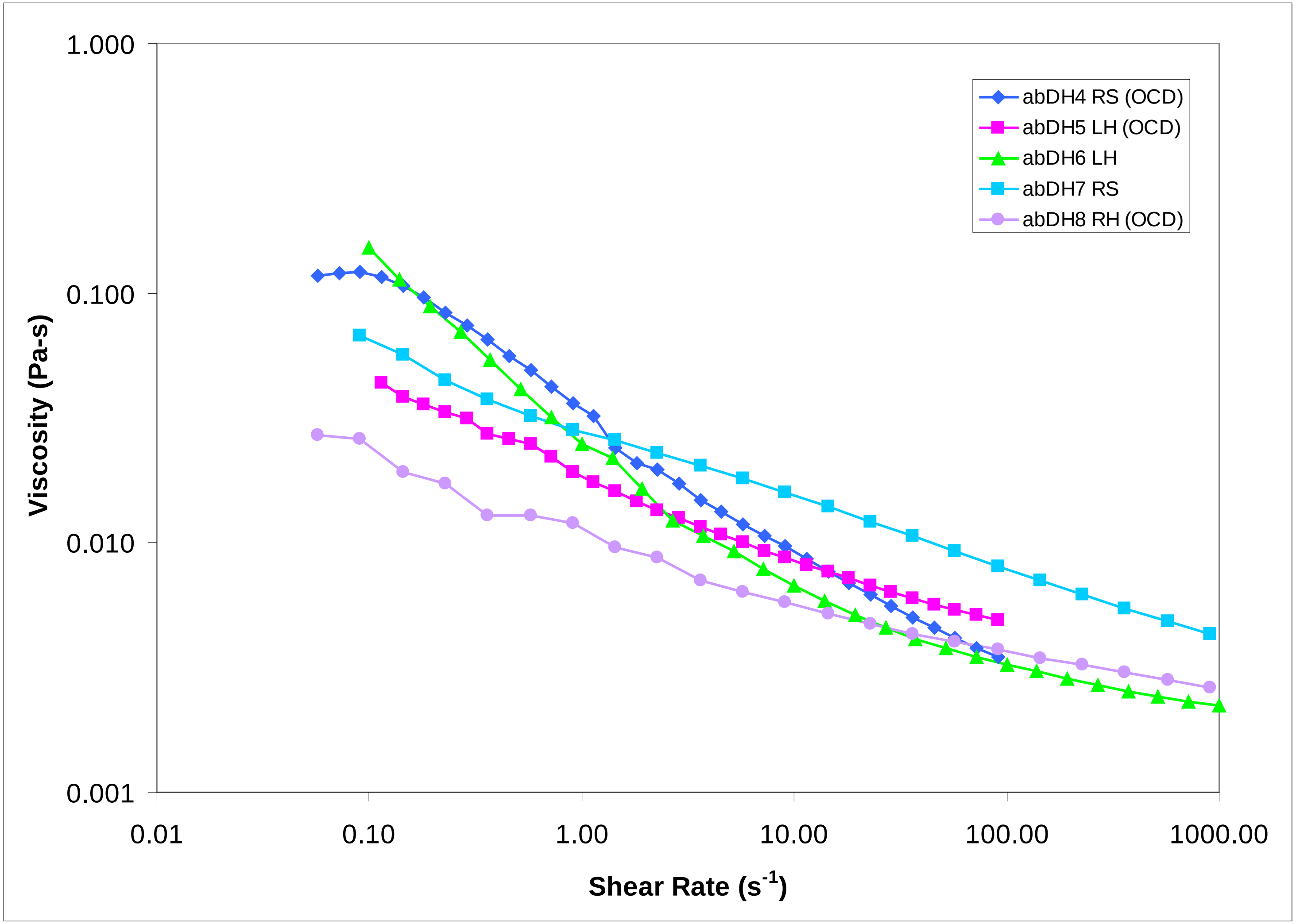
c = 0.48



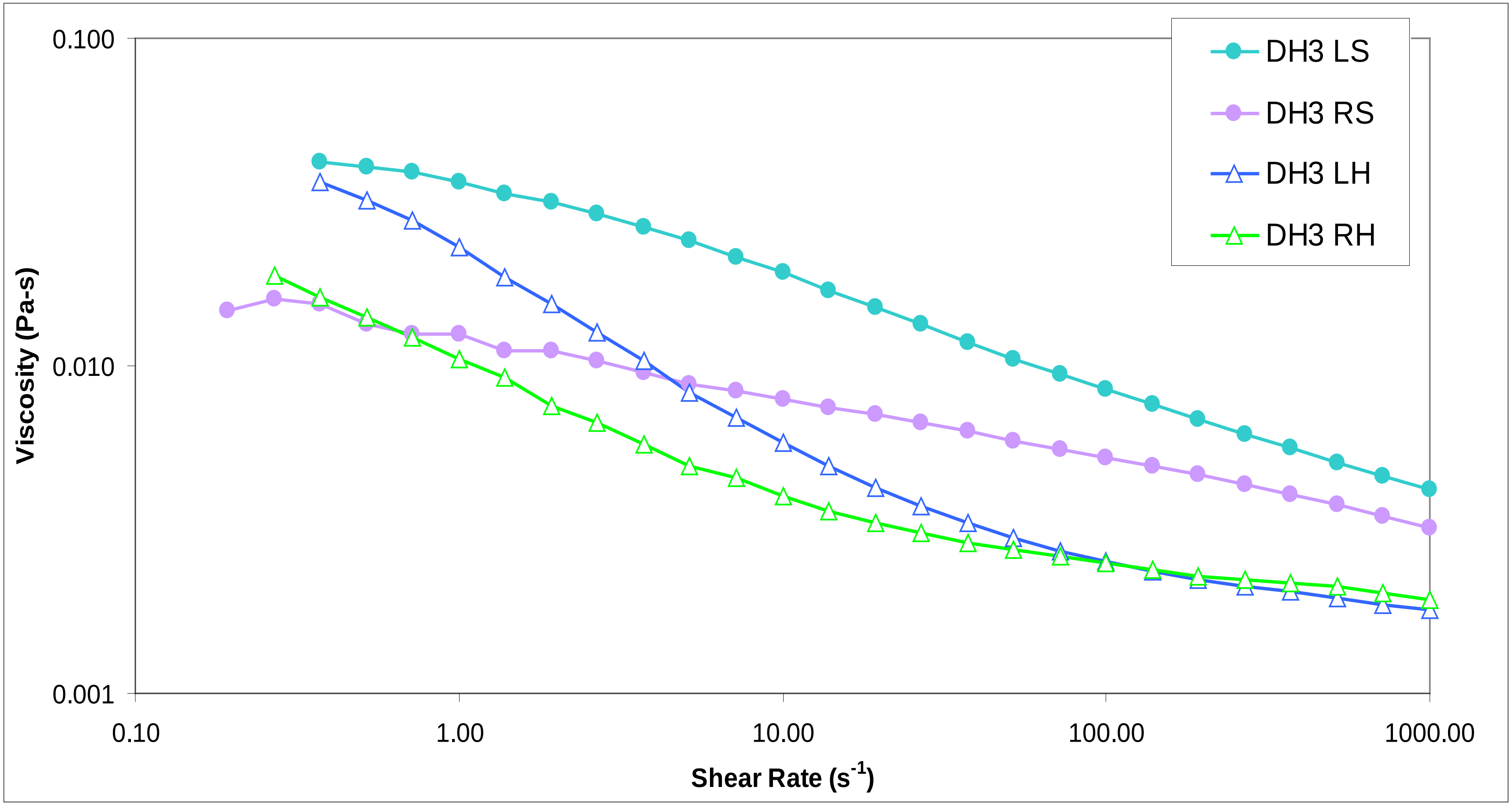
Steady Shear: Hocks



Steady Shear: Abnormal Joints



Steady Shear: DH3



Synovial Fluid Results Summary

- Molecular characterization
  - Mw range 2,000,000 to 6,500,000 Da
  - Concentration range 0.11 to 0.84 mg/ml
  - High standard deviation requires some technique refinement
- Rheological characterization
  - Upturn at low shear rates indicates aggregations
  - Similar viscosity range to HA at 2.5 mg/ml

Intra-articular Study

Intra-articular study: Introduction

- Viscosupplementation
  - Intra-articular (IA) injections
  - Intravenous (IV) treatment (equine)
  - Oral supplements sold (not FDA approved)
- Originally designed to boost mechanical properties of synovial fluid
  - Lasts up to 6 months
  - Lifetime ~96 hours in horses
- HA must play biochemical role in joint

Viscosupplementation

- Mechanism may depend on Mw of supplements
  - Hydrodynamic volume of HA effects which molecules can enter synovial cavity
  - HA may form complexes with other molecules
  - HA may interact with cell receptors
  - Injected HA may protect endogenous HA and stimulate production of more
- Understanding HA role in joints could contribute to development of new treatments

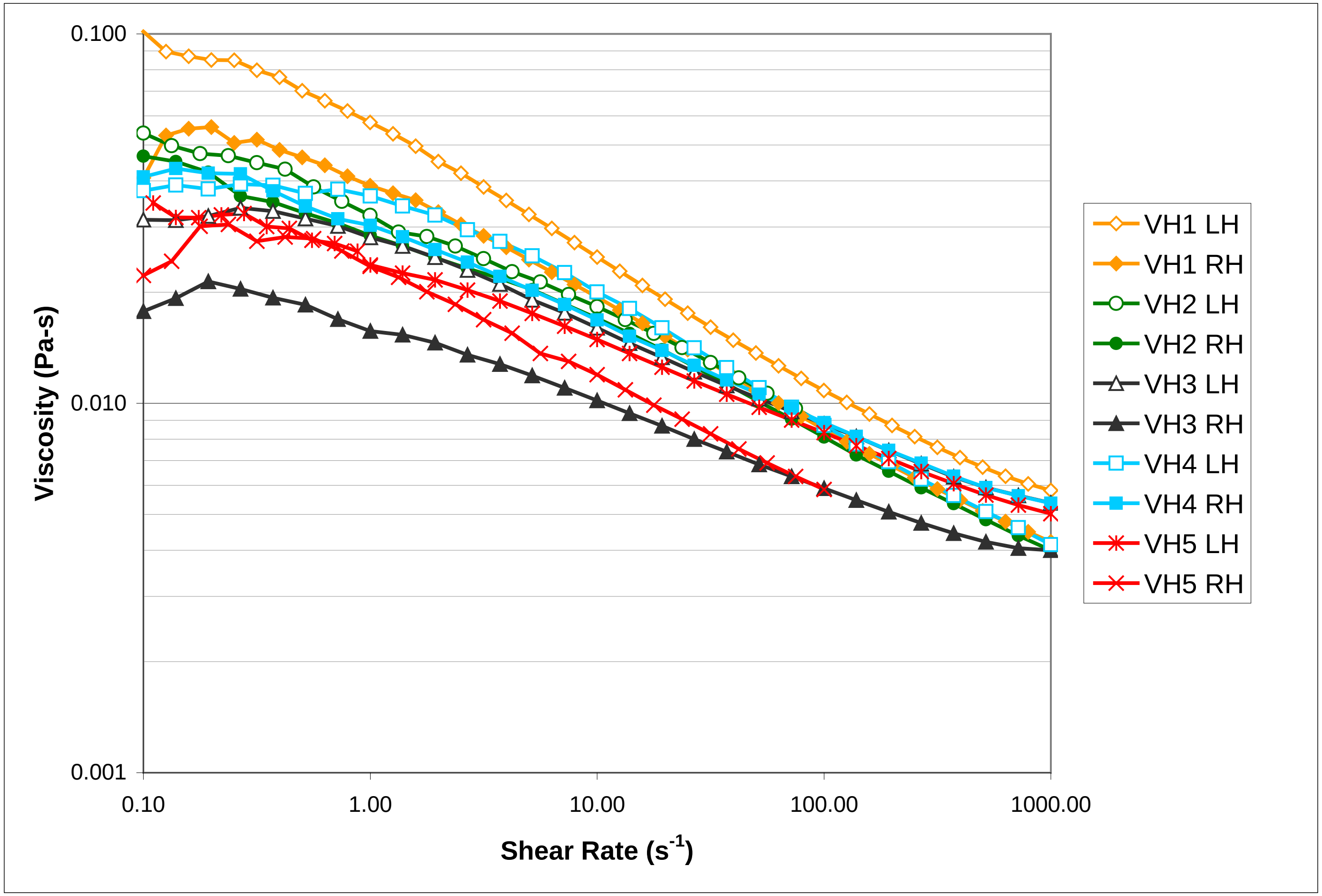
Intra-articular Study: Methods

- Five horses divided into three groups
  - Treatment Group (4 hocks)
    - Received 2 ml HA supplement, Hyvisc
      - Mw 1.75×10^6 Da
      - 11 mg/ml
  - Negative Control Group (3 hocks)
    - Received no injection
  - Positive Control Group (3 hocks)
    - Received 2 ml of sterile Lactated Ringers Solution (LRS)

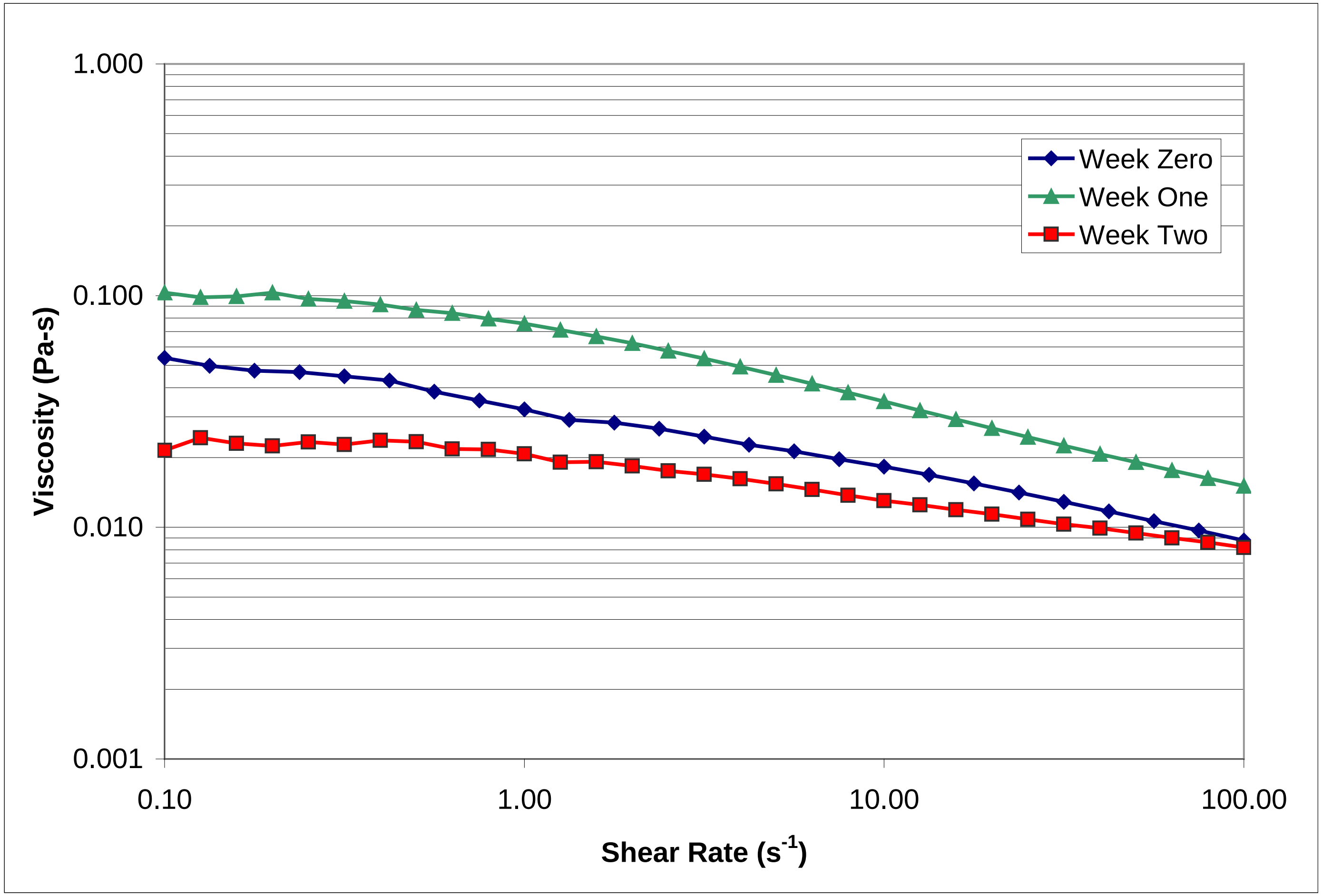
Intra-articular study: Methods

- Synovial fluid samples taken before treatment (baseline), one, and two weeks after
- Horses monitored daily for signs of infection
- Rheological characterization
  - Note: SEC-MALLS not available at time of study

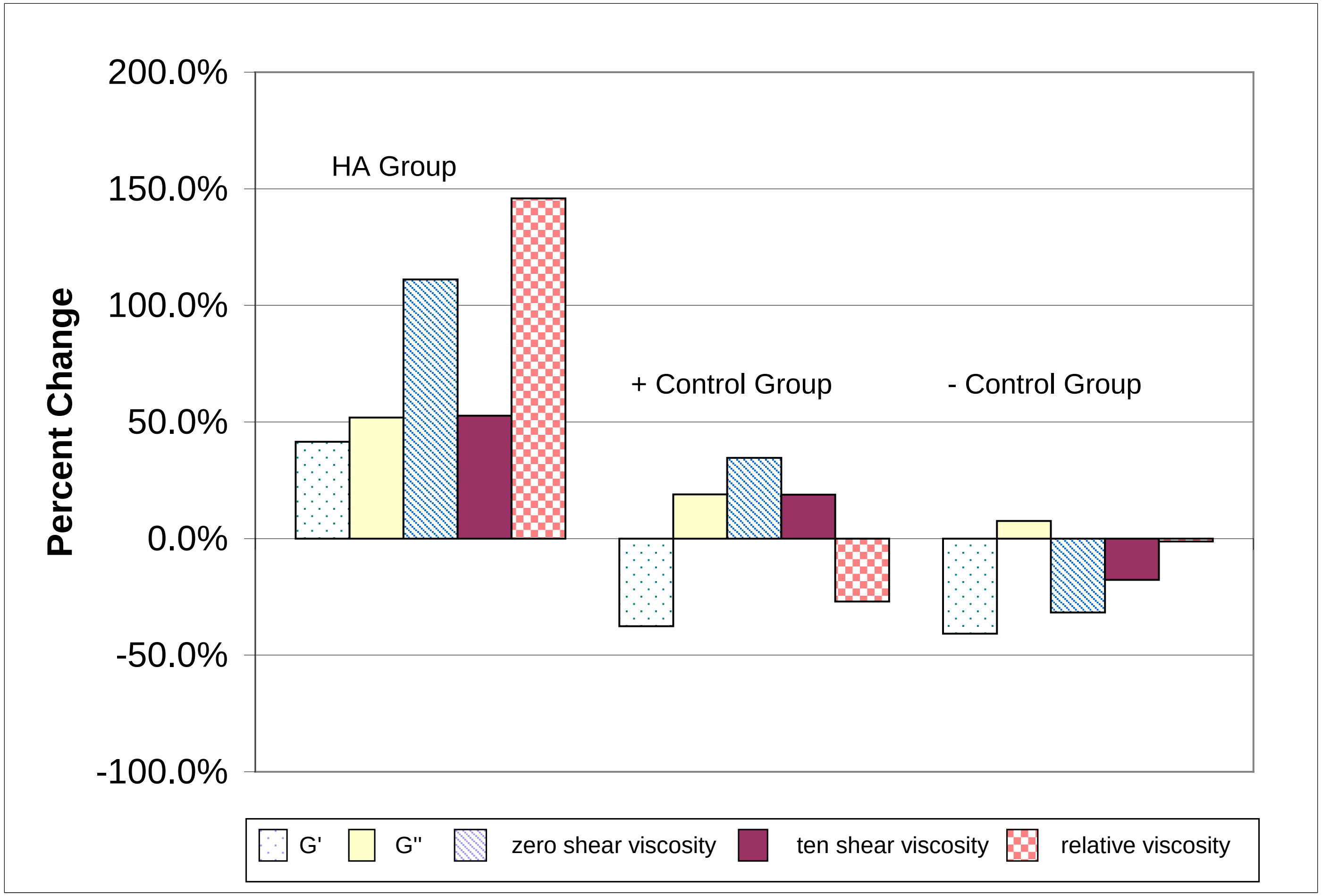
Steady Shear: All Hocks (Baseline)



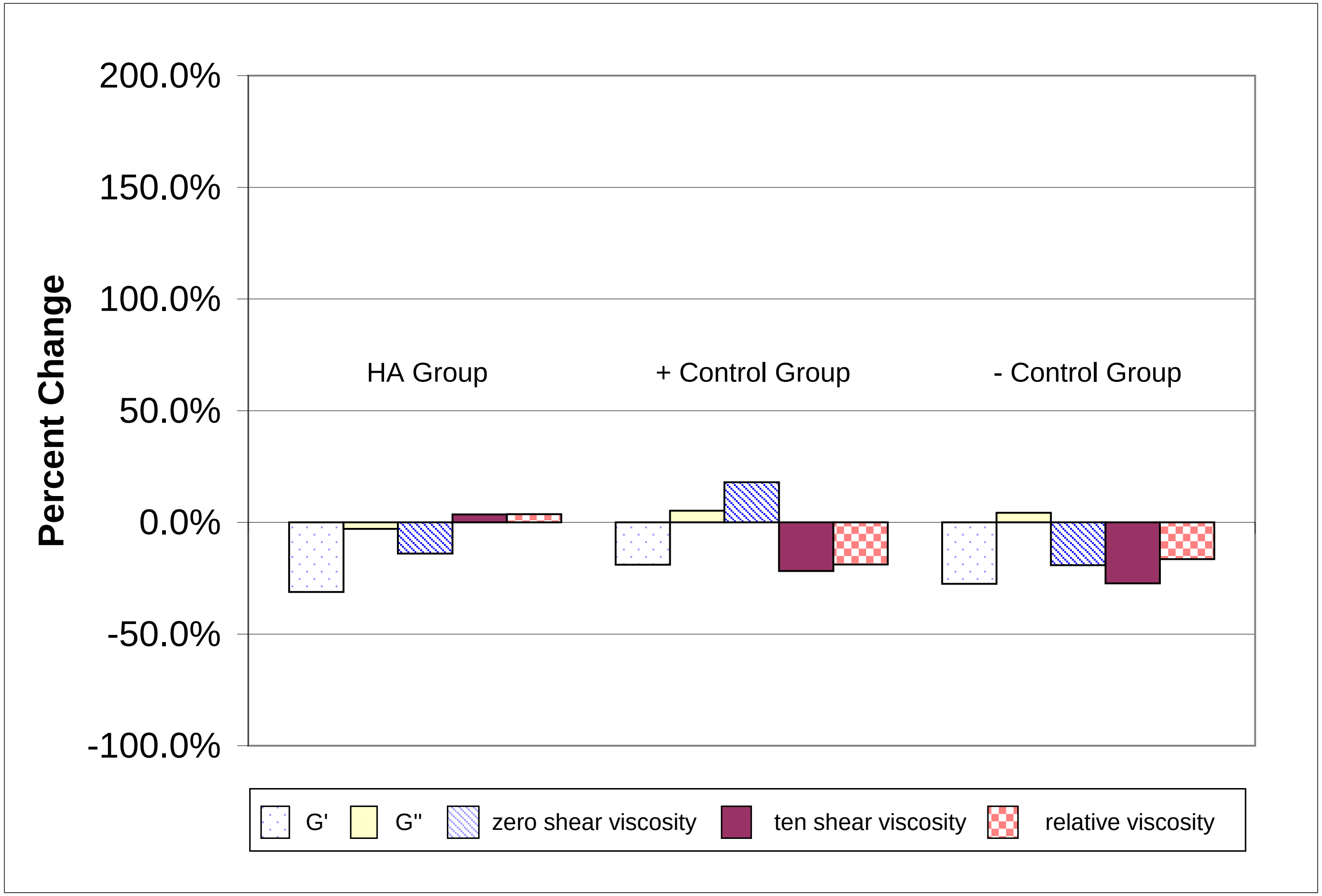
Results: Steady Shear Resuts of one Experimental Hock



Average Change in Viscoelastic Properties One Week after Treatment



Average Change in Viscoelastic Properties Two Weeks after Treatment



Summary of Intra-articular Study

- Sodium hyaluronate supplementation has a positive affect on the rheological properties of synovial fluid one week post-treatment
- Removing synovial fluid after one week made all hocks “negative controls”

Conclusions

- Sodium hyaluronate characterization
  - Molecular and rheological characterization was completed on sodium hyaluronate
  - HA behaves as a random coil in PBS
- Synovial fluid characterization
  - Mw $2x10^6$ to $6.5x10^6$ Da
  - HA concentration 0.11 to 0.84 mg/ml
  - HA appears to form aggregates
- Intra-articular study
  - HA injection has a positive effect on viscoelastic properties of synovial fluid one week after injection

Future Work

- Investigate repeatability issue of SEC-MALLS with synovial fluid
  - Treat synovial fluid with protease prior to analysis
  - Study effects of different sized filters
- Perform more studies on live horses
  - Use SEC-MALLS to quantify changes in synovial fluid after IA injection
  - Explore effects of IV application of HA

Acknowledgements

- Dr. Skip Rochefort, OSU ChE Dept
- Dr. Jill Parker, DVM, OSU College of Veterinary Medicine
- Sara Tracy
- Heidi Schmidt
- Committee Members

Sara Tracy's horse

Thank you