EFFECT OF COMPOSITION ON PERIPROSTHETIC FLUID RHEOLOGY AND FRICTION IN TOTAL KNEE ARTHROPLASTY

By

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ABSTRACT

The present thesis investigates the friction properties of total knee replacement components using lubricating fluids of different composition and rheology. The first stage of the thesis involves characterization of the rheological properties of hyaluronic acid (HA)/bovine calf serum (BCS) solutions. With increased concentration and molecular weight of HA, higher viscosities and more pronounced shear thinning in steady shear flow was observed. In dynamic oscillatory flow, the elastic character of the solutions became more pronounced and the cross-over frequency decreased upon increasing concentration and molecular weight of HA.

The second part of the thesis involves the determination of a method by which the coefficient of friction is estimated using a linear reciprocating wear testing machine with spherical metal indenters articulating on flat UHMWPE samples and deionised (D.I.) water lubrication. A characteristic periodic pattern in the friction behaviour was observed and the coefficient of friction was computed by calculating the average of 30 points about the midpoint between reversals and using the average of three cycles.

The third part of the thesis involves the investigation of the effect of fluid composition on the coefficient of friction in knee replacement components. With increased concentration of HA in solution (0-1mg/mL), the coefficient of friction of HA/BCS solutions decreased. However, with HA/BCS solutions of higher HA concentrations (above 1mg/mL), no

significant difference in the coefficient of friction was observed. Similar behavior was observed with HA/Albumin solutions. With HA/ D.I. water solutions, the coefficient of friction was almost identical irrespective of the concentration of HA in solution. Significant difference in the rheological properties of HA/D.I. water solutions did not affect the coefficient of friction. The Stribeck analysis revealed that the coefficient of friction with HA/BCS and HA/D.I. water lubrication was not governed by hydrodynamic conditions.

The fourth part of this thesis involved characterization of lubricating fluids before and after friction testing. Absorbance and dynamic light scattering measurements of HA/BCS solutions and of HA/Albumin solutions indicate a rise in turbidity and the presence of larger size particles in these solutions after friction testing. The ninhydrin test confirmed the presence of protein in the precipitates obtained following testing.

CO-AUTHORSHIP

This thesis contains chapters that present material that have been published in the form of journal articles as well as material that is under review as a technical communication. The complete citations for these papers and the chapters in which they appear are provided below:

Chapter 2: Fam, H., Bryant, J.T. and Kontopoulou, M. (2007) "Rheological properties of synovial fluids: A Review", *Biorheology*, 44 (2): 59-74.

Chapter 3: Fam, H., Kontopoulou, M., Bryant J.T. (2009) "Effect of concentration and molecular weight on the rheology of hyaluronic acid/bovine calf serum solutions", *Biorheology*, 46 (1): 31-43.

Chapter 4: Bryant, J.T., Fam, H., and Kontopoulou, M. (2009) "Method for friction determination in reciprocating wear tests", Technical Communication, *Wear*, Under Review

All three papers were co-authored by Dr. Tim Bryant and Dr. Marianna Kontopoulou. All of the remaining work and manuscript preparation was performed by the author. All manuscripts were reviewed by Dr. Tim Bryant and Dr. Marianna Kontopoulou prior to submission for publication.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

NOMENCLATURE

Symbols

η	viscosity
$\dot{\gamma}$	shear rate
ω	angular frequency
τ^*	stress vector
τ'	viscous component of shear stress
τ''	elastic component of shear stress
G'	storage modulus
G''	loss modulus
G^*	complex modulus
η^*	complex viscosity
η'	viscous component of the complex viscosity
η''	elastic component of the complex viscosity
η_o	zero shear rate viscosity
λ_r	relaxation time
θ'	viscometric relaxation time
$\dot{\gamma}_c$	shear rate at the onset of shear-thinning
n	flow index

$\eta_{\dot{\gamma}=300}$	viscosity at $\dot{\gamma} = 300$
$\overline{M}_w$	weight average molecular weight
f_c	cross over frequency
G_c	cross over modulus
$\tan\delta = \frac{G''}{G'}$	loss tangent

$\tau^* = \tau' - i\tau''$

$\eta^* = \eta' - i\eta''$

$G' = \eta'' \omega$

$G'' = \eta' \omega$

Abbreviations

HA	hyaluronic acid or hyaluronate
RA	rheumatoid arthritis
DJD	degenerative joint disease
THR	total hip replacement
TKA	total knee arthroplasty
TKR	total knee replacement
BCS	bovine calf serum
D.I.	deionised

CHAPTER 1

INTRODUCTION

Synovial joints are the bearings responsible for mobility in skeletal systems [1] . Synovial fluid is enclosed within the synovium; the tissue that surrounds the joint space [2] (Figure 1.1).

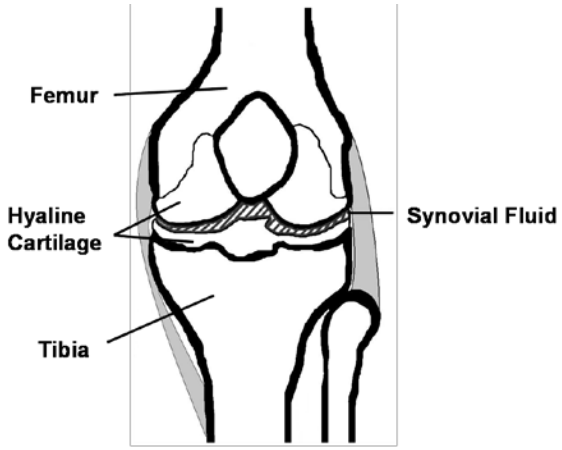

Figure 1.1 Synovial fluid in a knee. The femur (thigh) and tibia (shin) are lined with hyaline cartilage at the ends. Synovial fluid interacts with this specialized connective tissue to produce a low-friction bearing at the joint.

In addition to lubrication of the articular cartilage surfaces, synovial fluid is the medium by which nutrition in the form of glucose [3] is supplied to cartilage, since cartilage is avascular (no blood vessels, nerves or lymph). Synovial fluid is a dialysate of blood plasma consisting of proteins, lipids, phospholipids, and ions [4]. Hyaluronic acid (HA), a glycosaminoglycan produced by the synovial lining cells is also present in synovial

fluid [4]. Commercially, HA is produced from rooster combs or from the bacterial fermentation of Streptococcus strains, where it is used in different medical applications such as eye surgery and viscosupplementation of arthritic joints [5].

Due to its viscous character, the study of the rheology of synovial fluid has been of considerable scientific and clinical interest, specifically how the rheological properties of synovial fluid are affected in different pathological states such as in joint disease with osteoarthritis and rheumatoid arthritis. Osteoarthritis or degenerative joint disease (DJD) is a joint disease characterized by erosion of articular cartilage which becomes soft, worn, and thinned, leading to pain and loss of function of primarily load bearing joints [6]. Rheumatoid arthritis (RA) is a systemic joint disease which affects connective tissue involving many joints and accompanied by thickening of articular soft tissue and erosion of articular cartilage leading to deformities and disability [6]. Both viscosity and elasticity are lowered in pathological synovial fluids from patients with osteoarthritis and RA due to both a decrease in HA concentration and molecular weight. While studies on fluid obtained from primary and revision joint replacements are limited, they indicate that HA is regenerated following prosthesis surgery, although at lower concentrations than observed prior to initial joint replacement.

Periprosthetic fluid, the fluid that bathes total joint replacements *in vivo*, also contains HA and recent findings indicate that its presence is responsible for the observed viscous

properties [7,8]. Total knee arthroplasty (TKA) is a surgical procedures widely administered to patients with osteoarthritis, rheumatoid arthritis, and joint trauma. The femoral component is typically made of a metal alloy such as Cobalt Chrome (Co-Cr) and the tibial component includes an insert typically made of UHMWPE (ultra-high molecular weight polyethylene) on a metal backing made of an alloy such as Co-Cr (cobalt-chrome) or titanium. Wear of the articulating joint surfaces is a major limitation of this procedure which results in particles that induce an immune response in the body. This results in bone resorption, loosening of the joint, and ultimately revision surgery [9].

Currently, the in *vitro* wear testing lubricant of orthopaedic implants is BCS, due to its resemblance in chemical composition to periprosthetic fluid. However, BCS does not contain HA, which would render its rheological properties more relevant to those of the actual periprosthetic fluid. Attempts have been made to develop a clinically relevant simulator lubricant with HA supplemented bovine serum [10]. Addition of 1.5 mg/mL HA to 50% bovine serum revealed an increased viscosity and shear thinning character, whereas Newtonian behaviour was observed in bovine serum alone [10]. Interestingly, higher wear rates were observed with the HA supplemented serum as opposed to lubrication with serum alone in knee simulator testing.

The rheological properties of HA/BCS in the context of TKA lubrication have not been thoroughly studied. The non-conforming nature of a knee replacement makes it difficult

to predict its tribological performance. In addition the speeds encountered in one gait cycle are variable [11] which leads to variable shear rates experienced by the lubricating fluid. Since normal synovial fluid and periprosthetic fluid are non-Newtonian [4], the viscosity varies with shear rate. Fluid viscosity affects the minimum film thickness which is critical in determining the mode of lubrication encountered by the joint [12].

Wear of knee replacement components has been a focus of studies directed toward optimizing the performance of these bearings [10,13-28]. Fewer studies have been undertaken to evaluate friction in these systems [29-31]. In particular, friction measurements could reveal the mode of lubrication occurring within joint prostheses.

Replacement of diseased or injured hip joints became common practice in the late 1950s, and recently knee replacement surgeries have conducted to restore joint function and to alleviate pain. In particular, the first joint system by Sir John Charnley aimed at low friction, however the wear of these bearings was high [32].

The focus of this thesis is aimed towards the understanding of the friction behavior of knee replacement bearing systems. The objectives of this thesis are the following:

- To Identify the effect of periprosthetic fluid composition on its rheology.
- Establish a method for friction determination in a reciprocating wear simulator.

- Determine the effect of periprosthetic fluid composition on friction in metal on UHMWPE systems.
- Determine the mechanism of friction behaviour in these systems.

The ultimate scope is to improve our understanding of the friction properties in knee replacements with the long term goal of improving joint replacement performance. The scope of this thesis is limited to metal on UHMWPE knee replacement systems.

CHAPTER 2

LITERATURE REVIEW

2.1 Chemical Composition of Synovial Fluid

Synovial fluid is a blood plasma dialysate [2,33], which contains hyaluronic acid, a polysaccharide produced by the synovial lining cells [2]. The name *hyaluronic acid* is derived from the Greek word *hyalos*, which means glassy and from the presence of uronic acid in the polymer [34].

The composition of synovial fluid in normal, osteoarthritis and RA fluids is depicted in Table 2.1. The protein content depends on whether the joint is healthy or diseased; the protein content in normal fluid is substantially lower than that of plasma [35,36]. However, in inflammatory and degenerative synovial fluid, the protein content increases [37,38], with that in the former becoming similar to plasma [35,39]. Albumin and γ-Globulin are the main proteins in normal synovial fluid and larger proteins such as fibrinogen, immunoglobulin IgM, and the metalloproteinase inhibitor, α_2-macroglobulin are found in very low concentrations [40]. Cytokines, collagen, enzymes, proteoglycans, and fibronectin are present in low amounts in normal synovial fluid and increase in concentration in degenerative and inflammatory arthritis [40]. In addition, the glycoprotein lubricin, found to exhibit lubricating properties, was isolated from diseased human knee synovial fluid [41] and from bovine synovial fluid [42]. Electrolytes such as

Na^+ and Cl^-, and small molecules such as urea and glucose are also present in normal synovial fluid [40]. Increased amounts of lipids were also found in synovial fluid [43] of patients with RA and osteoarthritis [44]. Bole [45] reported that in normal synovial fluid the lipid concentration is considerably lower than that of plasma, while in RA synovial fluid, it is as high as 40-60% of plasma concentration. Phospholipids and cholesterol were found in very small amounts in normal synovial fluid and neutral lipids such as triglycerides and esterified cholesterols were non-existent [45]. Lipoproteins are also present in very low amounts in normal synovial fluid [46], while they exist in increased quantities in pathological synovial fluids with amounts in RA exceeding those found in osteoarthritis [47-49].

Table 2.1 Composition of normal, osteoarthritis, and RA synovial fluid from various reports.

	Normal		**Osteoarthritis**		**RA**	
	Value	Ref.	Value	Ref.	Value	Ref.
HA $\overline{M}_w$ (MDa)	6.3-7.6	[50]	1.06-3.48	[51]	3.2-6.8	[50]
HA (mg/mL)	2.50-3.65	[52]	1.07-2.60	[52]	0.39-2.19	[52]
	1.45-2.94	[53]	0.32-3.61	[55]	0.70-3.74	[55]
	2.0-4.0	[40]	1.24-2.22	[54]	0.37-1.88	[53]
	0.35-4.22	[54]			0.19-1.26	[50]
					0.35-2.41	[54]
					0.8-1.7	[56]
Protein (mg/mL)	10.4-15.8	[53]	17.0-56.8	[55]	31.6-66.2	[55]
	12-30	[2]			19.8-49.5	[53]
	13.1-21.3	[36]				
Phospholipid (mg/mL)	0.13-0.15	[48]	0.26-0.98	[48]	0.40-1.40	[48]
Total Cholesterol (mg/mL)	0.07-0.08	[48]	0.04-1.69	[48]	0.76-1.30	[48]
Triglycerides (mg/mL)	0	[48]	0.12-0.59	[48]	0.17-1.00	[48]

Cellular components present in small amounts in normal synovial fluid include leukocytes: lymphocytes, monocytes, synovial lining cells, and polymorphonuclear cells [2]. The total leukocyte count and the differential count of polymorphonuclear cells increase in non-inflammatory and inflammatory joint disease with higher counts in the

latter [2]. Clinically, synovial fluid can be aspirated to diagnose joint disease and typical examination tests include: color, clarity, viscosity, clot formation, and cytology [57].

Given the importance of HA and its postulated influence on the rheological properties of synovial fluid, the section that follows provides a detailed description of its properties and rheology.

2.1.1 Properties and rheology of HA protein complex

HA which was first isolated from vitreous humor [58] is a glycosaminoglycan consisting of alternating N-acetyl D-glucosamine and D-glucuronic acid units [58] (Figure 2.1). The glucuronic acid and the N-acetyl glucosamine are linked $\beta(1\rightarrow 3)$, while the N-acetyl glucosamine and the glucuronic acid are linked $\beta(1\rightarrow 4)$ [59].

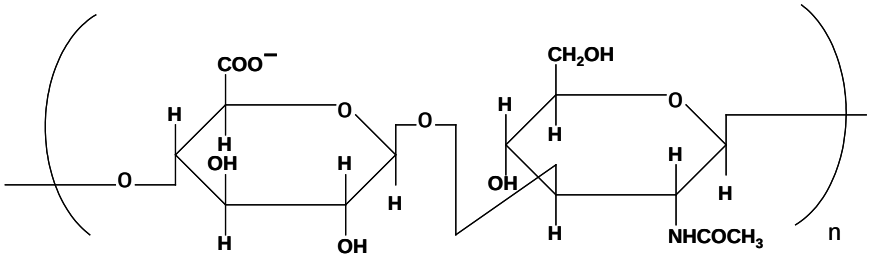

Figure 2.1 Structure of HA after Krause *et al*. [60].

Ogston and Stanier [61] proposed that HA is chemically bound to proteins, forming an HA protein complex that affects the non-Newtonian character of synovial fluid. When the protein of ox-synovial fluid was hydrolyzed with papain, a proteolytic enzyme in the

presence of EDTA, a significant drop in the viscosity of the complex resulted [62]. This was also confirmed by studies in which longer incubation times led to further reduction in the viscosity of acute traumatic synovial fluid [63].

However, studies by Ogston and Sherman [64] showed that EDTA alone degraded the complex resulting in a reduction of the viscosity at all shear rates. They concluded that the physiochemical properties of the complex were not dependent on the protein component; there was no change in the viscosity of the complex upon incubation with or without proteolytic enzymes, namely trypsin and chymotrypsin. The results were not evidence against the presence of a HA protein complex but were evidence that the protein component did not influence the rheology of the complex. HA is bound to protein in small quantities (about 2% protein is present [65,66]). Thus, the existence of proteins does not affect the viscosity of HA [67]. The destructive effects on viscosity, resulting from proteolysis of protein-polysaccharide complexes is not observed in the HA-protein complex [66,68], and hence HA is the major determinant of complex rheology.

In order to determine the extent to which the protein complex affects the viscoelastic properties of HA, the protein component must be separated from the complex, which is difficult since separation can also lead to HA degradation [69]. Altmann and Zeidler [70] separated HA from protein in healthy ox synovial fluid and found that two different apparent viscosity vs. shear rate curves were obtained for the isolated HA and for the

native synovial fluid; the zero shear rate viscosity decreased, and the extent of shear-thinning was lower for the isolated HA curve. When the protein was recombined with HA, neither the viscosity vs. shear rate curve nor the normal forces of the original synovial fluid were reproduced. The authors suggested that an irreversible change of the viscosity vs. shear rate curve and of normal forces occurred as a result of the isolation of HA by the method they used namely: caesium chloride-density-gradient ultracentrifugation. This method degrades the polymer leading to permanent changes in viscoelasticity.

HA is likely the primary determinant of synovial fluid viscoelasticity since enzymatic treatment by an HA degrading enzyme led to the loss of both viscous and elastic properties of synovial fluid [71]. Upon enzymatic degradation of pathological synovial fluids a substantial decrease in both the viscous and the elastic components of shear stress as well as viscosity were observed [71].

2.1.2 Structure and rheology of HA aqueous solutions

HA in aqueous solutions exists as an extended random coil at low concentrations (< 1 mg/mL) [72], allowing for free movement of the polymer chains [73]. At high concentrations (> 1 mg/mL), a transient entanglement network arises where molecules entangle with each other and then disentangle after a period of time [73]. This network is stabilized by hydrogen bonding and non-covalent intermolecular associations [73,74]. The network properties of HA at high concentration affect the viscoelasticity of the

solution [75], giving rise to enhanced viscosity and pronounced non-Newtonian behaviour, manifested by a higher extent of shear thinning [60,74,76].

Similar effects have been noted as the molecular weight of HA in solution is increased for solutions of the same concentration [75]. Solutions of HA of molecular weight of 3×10^6 Da showed increased magnitudes of the viscous and elastic components of the complex viscosity, η' and η'', respectively with frequency compared to solutions with HA of molecular weight of 0.5×10^6 Da at the same concentration [77]. However, the increase was more pronounced for the elastic component, η''.

The effect of molecular weight of HA on the dynamic moduli G' and G'' was investigated by Kobayashi *et al.* [78]. For the higher molecular weight HA solutions, G' exceeded G'' at the high frequency range, while the opposite was true at the low frequency range. This distinct transition from viscous to elastic behaviour was also observed by Gibbs *et al*. [79] for solutions of HA at a molecular weight of 2.8×10^6. It was postulated that the relaxation mechanism involves viscous flow preceded by a breakdown of a hydrogen-bonded network [79]. Kobayashi *et al.* [78] indicated that a transient network is formed by entanglements of HA chains at high molecular weight and these entanglements were absent for HA at low molecular weight.

Similar trends were found in studies by Ambrosio *et al*. [80], who indicated that in the case of high molecular weight HA (1.2×10^6 Da) a network structure is formed from entanglements, as opposed to the low molecular weight HA (0.15×10^6) where entanglements are absent. The dynamic moduli G' and G'' exhibited cross-over for the higher molecular weight HA, in contrast to the low molecular weight HA that behaved like a viscous fluid throughout the range of frequencies examined [80]. Also, for the higher molecular weight HA, a lower cross-over frequency was observed at higher concentrations of HA. The authors postulated that the cross-over frequency corresponds to the polymer chains' disentanglement rate that is a function of their mobility, which is in turn affected by both the molecular weight and the concentration of HA in solution.

The rheology of HA in solution could also be affected by the presence of HA of lower molecular weight [81]. At physiological pH and ionic strength, HA polymer solutions displayed frequency dependence of the dynamic moduli G' and G'' typical of transient polymer networks; a pseudoplastic plateau was observed at the high frequency region. Addition of HA segments (~60 disaccharide units) at equal concentration to the HA polymer solution led to a substantial decrease in both moduli and the pseudoplastic plateau was lost. Such behaviour was attributed to competitive inhibition where short chains are able to disrupt intermolecular network formation. This phenomenon was not observed by Fujii *et al*. [82] where addition of an equal concentration of HA segments (50 disaccharide units) to HA physiological saline solution had no effect on the frequency

dependence of the dynamic moduli. It was postulated that shorter segments do not block the active sites of longer HA chains and hence do not prevent the association of these longer HA chains to increase the viscoelasticity. Addition of longer chain segments increased both moduli by increasing the entanglement of HA.

In addition, the presence of ions such as Na^{+}, Ca^{++} and K^{+} and small molecules such as urea and glucose could also affect the rheology of HA solutions. In studies by Kobayashi *et al*. [78], addition of $CaCl_2$, KCl and NaCl decreased the dynamic moduli of HA solutions. This was attributed to the shielding of electrostatic repulsions of the carboxyl groups in HA molecules by the cations in these salts [78]. Similar results were observed by Fujii *et al*. [82] and by Mo *et al.* [83] when NaCl was added to HA solutions. Addition of urea and guanidine hydrochloride to osteoarthritic synovial fluid led to a fall in the viscosity over the shear rate range tested. This was attributed to the hydrogen bond breaking effect of urea and guanidine hydrochloride on HA in osteoarthritic synovial fluid. Guanidine hydrochloride decreased both the elastic and viscous moduli of sodium hyaluronate (HA) solutions and the cross-over frequencies increased as the concentration of guanidine hydrochloride increased [83]. This was ascribed to the shielding of electrostatic repulsion of sodium hyaluronate solutions by guanidinium ions. Conversely, glucose increased both the storage and the loss moduli of HA solutions due to the creation of new hydrogen bonds that strengthen the entanglement network of HA [78].

2.2 Viscoelastic properties of normal and pathological synovial fluids

2.2.1 Viscometric properties

Normal synovial fluid is a non-Newtonian fluid with shear-rate dependent viscosity [84-86]. Studies of healthy and pathological synovial fluids have been performed with the aim to obtain the rheological parameters characteristic to a specific fluid pathology from measured viscosity vs. shear rate curves. Rainer and Ribitsch [87], who measured the viscosity of normal synovial fluid samples as a function of shear rate, reported their zero shear rate viscosities, η_o and the ratio $\frac{\eta_o}{\eta_{\dot{\gamma}=300}}$ (which they used as a measure of the extent of shear-thinning of the fluid). The range obtained for η_o was 6-175 Pa s, and the range of $\frac{\eta_o}{\eta_{\dot{\gamma}=300}}$ was 70-250 (Table 2.2).

Table 2.2 Rheological parameters for normal and pathological fluids. Data from Schurz and Ribitsch [88] and from Rainer and Ribitsch [87] were obtained at 25°C. Data from Anadere *et al*. [89] and Safari *et al*. [90] were obtained at 23°C and 27°C respectively. RA fluids designated as s.n. indicate seronegative and as s.p. indicate seropositive.

	Normal		**Osteoarthritis**		**Inflammatory**	
	Value	Ref.	Value	Ref.	Value	Ref.
η_o (Pa.s)	6-175 1-40	[87] [88]	0.1-1	[88]	0.004-0.07	[88]
$\frac{\eta_o}{\eta_{\dot{\gamma}=300}}$	70-250 100	[87] [88]	5-40	[88]	1-4	[88]
η' At 2 Hz (mPa.s)	366-513	[90]	31.0 ± 15.0	[89]	19.5 ± 7.2 (RA. s.n.) 20-38 (RA. s.n.) 9.91 ± 3.8 (RA. s.p.) 5-15 (RA s.p.)	[89] [90] [89] [90]
η'' At 2 Hz (mPa.s)	789-1406	[90]	36.33 ± 28.0	[89]	11.2 ± 6.8 (RA s.n.) 11-25 (RA s.n.) 3.4 ± 4.1 (RA s.p.) 0.05-9 (RA s.p.)	[89] [90] [89] [90]
λ_r (s)	0.65-5.82	[90]	-		0.022-0.03 (RA s.n.) <0.022 (RA s.p.)	[90]
θ' (s)	40-100	[88]	8-20	[88]	0.02-1	[88]

Earlier studies on normal and RA fluid by Johnston [91] showed that, compared to normal post mortem synovial fluid, the viscosity of RA synovial fluid is considerably

lower at the measured shear rates. Bloch and Dintenfass [92] found that synovial fluid from RA is Newtonian; the viscosity was of the order of 0.01 Pa s (10 centipoise). Shear-thinning behaviour is characteristic of normal synovial fluid [93] and osteoarthritic synovial fluid [94], while Newtonian behaviour is characteristic of RA fluid [93].

These findings were corroborated by Cooke *et al*. [95], who determined the rheological properties for synovial fluid from normal, osteoarthritic, and rheumatoid arthritic human joints. For the range of shear rates tested, the viscosity was found to be higher for normal as opposed to osteoarthritic fluid, which in turn was higher than that of RA fluid. Similar observations were reported for the three pathologies by Schurz and Ribitsch [88] (Figure 2.2) and for osteoarthritic and RA fluids by Davies and Palfrey [96]. In pathological synovial fluids, η_o and the extent of shear-thinning are lower than in normal synovial fluids [88,97,98].

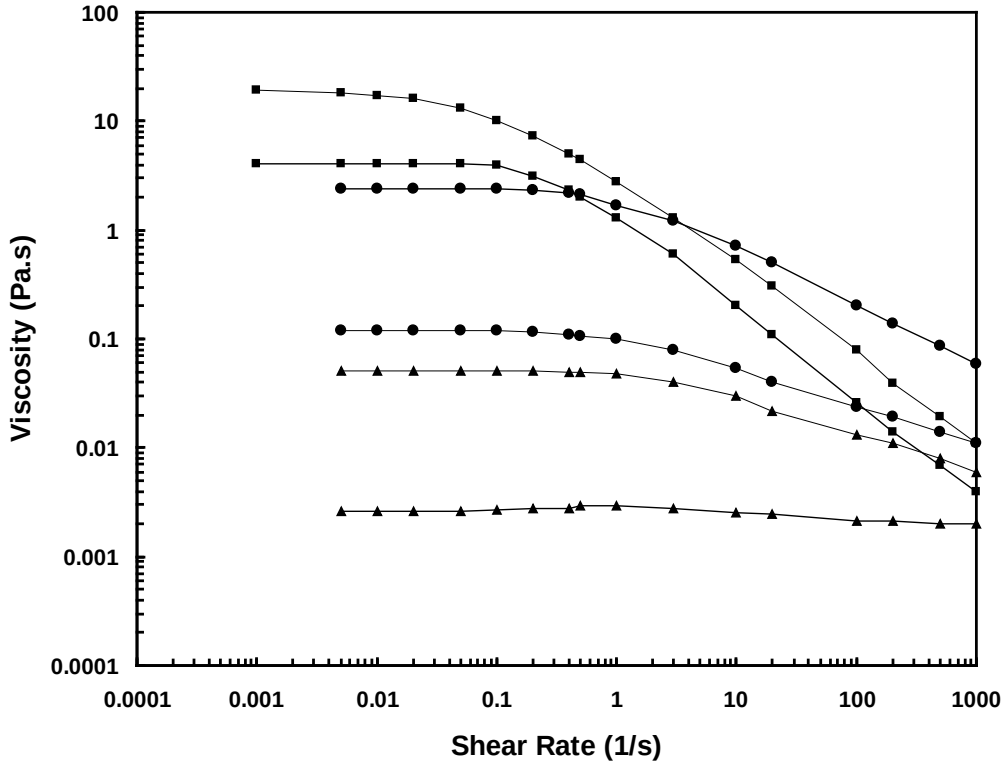

Figure 2.2 Viscosity ranges for healthy, degenerative, and inflammatory synovial fluids after Schurz and Ribitsch [88] . Squares, circles and triangles define the upper and lower boundaries of healthy, degenerative, and inflammatory synovial fluids respectively.

Normal fluids have the highest η_o and $\frac{\eta_o}{\eta_{\dot{\gamma}=300}}$ followed by degenerative fluids, and lastly by inflammatory synovial fluids [88]. The range of η_o obtained for normal, degenerative, and inflammatory synovial fluids respectively are: 1-40, 0.1-1, and 0.004-0.07 Pa.s. respectively (Table 2.2) [88]. The values for $\frac{\eta_o}{\eta_{\dot{\gamma}=300}}$ for normal, degenerative, and

inflammatory synovial fluids respectively are: 100, 5-40, and 1 - 4 (Table 2.2) [88]. Similar trends were also found by Rainer *et al.* [98]. It is important to note that the difference in viscosity obtained from the different synovial fluid pathologies is only distinct at the low shear rate region. At high shear rates, the viscosities of normal and pathological fluids are closer in magnitude [95,99] due to the enhanced shear-thinning character of the former.

Based on steady-shear viscosity data, Schurz and Ribitsch [88] calculated a "relaxation time", $\theta' = \frac{1}{\dot{\gamma}_c}$, where $\dot{\gamma}_c$ is the shear rate at the onset of shear-thinning. The ranges obtained for θ' were: 40-100 s, 8-20 s and 0.02-1 s for normal, degenerative and inflammatory synovial fluids respectively (Table 2.2) [88].

2.2.2 Viscoelastic properties

The viscoelasticity of synovial fluid was first investigated by Ogston *et al*. [100] who concluded that synovial fluid possesses elastic properties. This was later confirmed by other researchers [101-103]. Myers *et al*. [69] determined the viscoelastic properties of normal synovial fluids from human knee joints at frequencies corresponding to different joint speeds. At low frequencies of oscillation, characteristic of slow joint motion, synovial fluid behaves as a viscous fluid, while at high frequencies, characteristic of rapid joint motion, it has an elastic-like response (Figure 2.3).

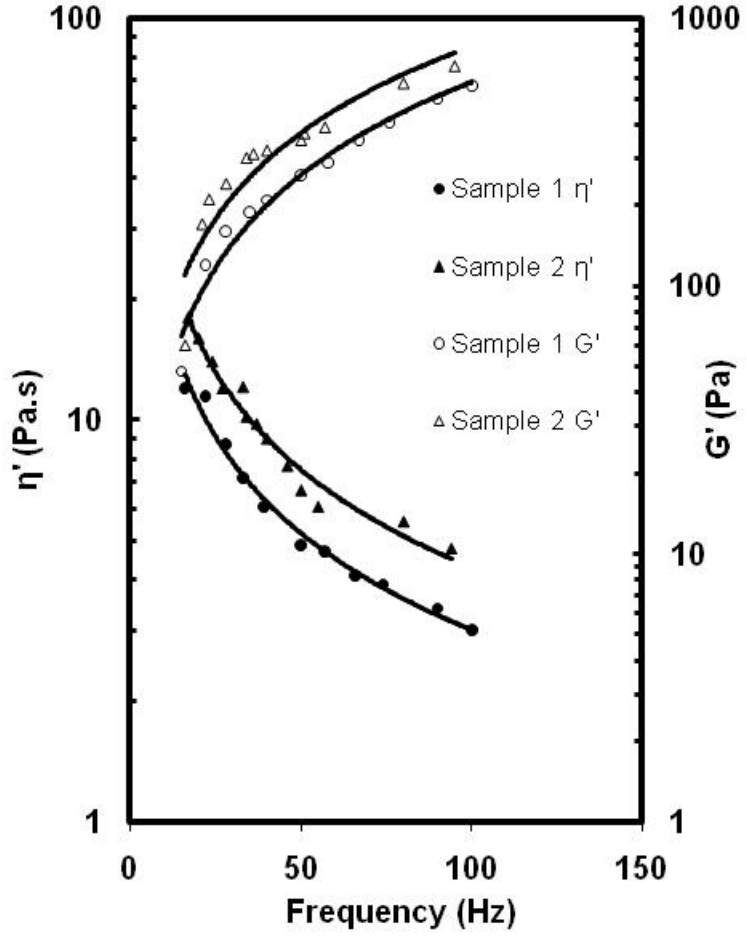

Figure 2.3 The dynamic viscosity and elasticity of two samples of synovial fluid after Myers *et al.* [69], with permission.

The dynamic moduli, G' and G'', were determined for "near normal" and diseased synovial fluid by Safari *et al*. [90] using oscillatory rheometry . As shown in Figure 2.4, for the "near normal" synovial fluid, G'' is higher than G' at very low frequencies. Cross-over occurs at a relatively low frequency (~ 0.02 Hz) and at higher frequencies, the

fluid exhibits elastic-like behaviour likely because it does not have enough time to dissipate energy [90].

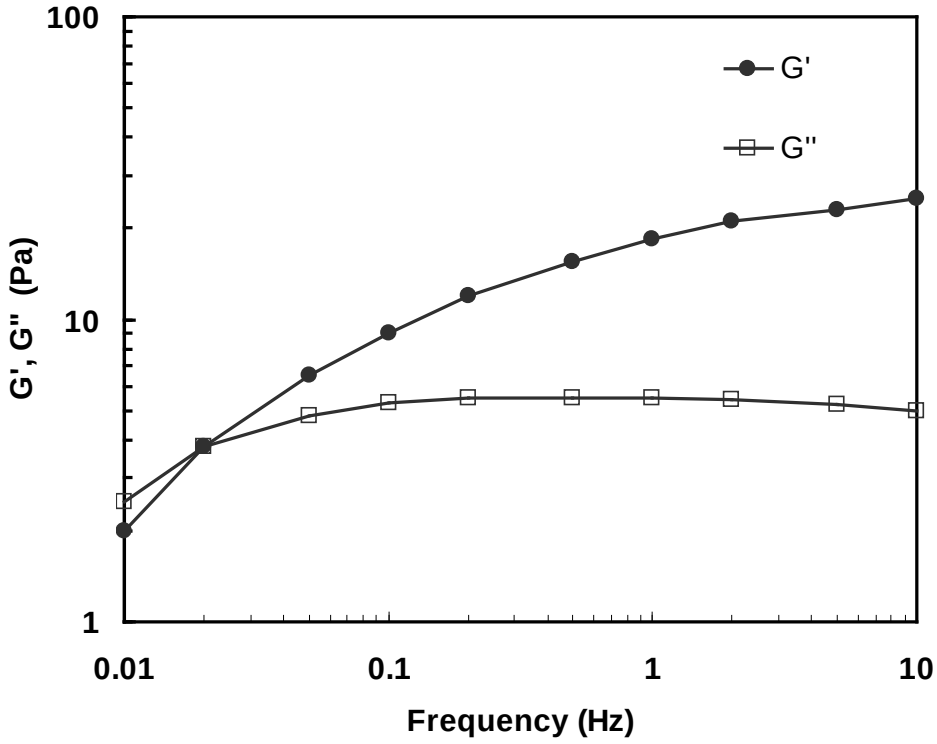

Figure 2.4 The dynamic moduli of "near normal" synovial fluid versus frequency at 27°C after Safari *et al.*[90] , with permission.

Pathological synovial fluids however, behave generally as viscous fluids [104] and their storage modulus G' is lower than that of healthy synovial fluids at frequencies above 0.1Hz [104]. Relaxation times, λ_r calculated from the cross-over frequency, $\omega_c = \frac{1}{\lambda_r}$,

where $G' = G''$, were longest for normal fluids, followed by fluids from patients with ligament defects and lastly by those from patients with RA seronegative, and RA seropositive. The corresponding ranges of values were 0.65-5.82 s, 0.59-0.89 s, 0.022-0.03 s, and <0.022 s respectively (Table 2.2). For one patient with RA seropositive, G' did not intersect G''; the authors concluded that at this pathological state, synovial fluid has virtually no elasticity. In patients with RA seropositive, the rheumatoid factor blood test is positive and in those with RA seronegative the test is negative. Patients with RA seropositive have a more severe disease course with more joint deformities, disability and inflammation. Similar trends were obtained by Balazs *et al*. [105] where no cross-over behaviour was observed for osteoarthritic synovial fluid as opposed to normal synovial fluid (Figure 2.5). Within normal fluids, the cross-over frequency of synovial fluid from an old patient was between walking and running frequency and was higher than that of synovial fluid from a young patient.

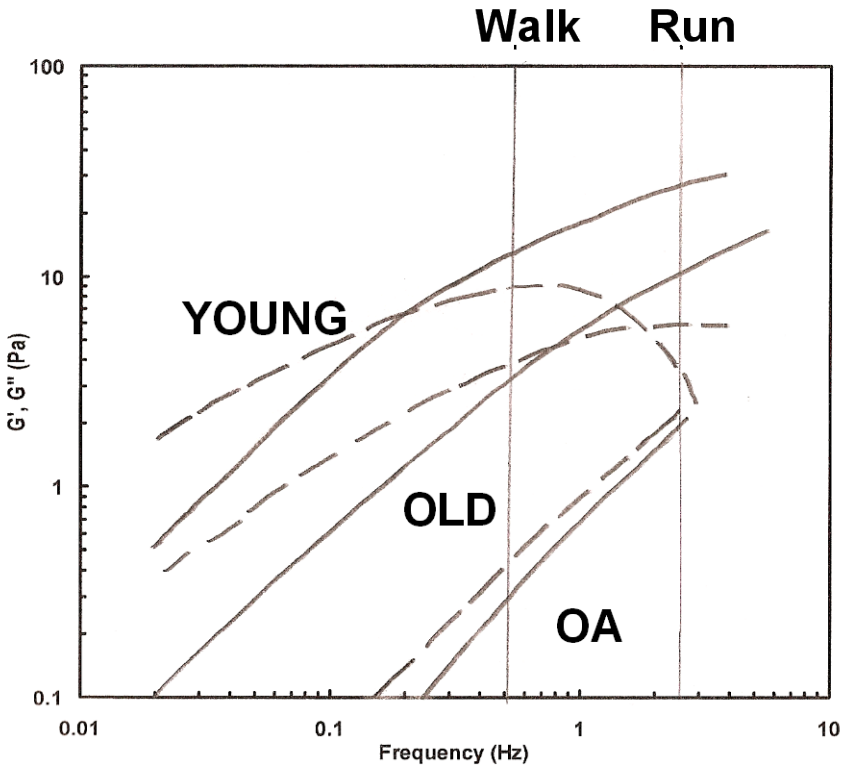

Figure 2.5 The dynamic moduli of synovial fluid from young normal, old normal and osteoarthritis patients after Balazs *et al*.[106].

Safari *et al*. [90] also reported that for the "near normal" synovial fluid group and for the synovial fluid group from patients with ligament defects, the mean values of η'' were higher than the mean values of η', while the opposite was true for RA seronegative and RA seropositive fluid groups at a frequency of 2 Hz. Similar findings were obtained by Anadere *et al*. [89] for the RA seropositive and RA seronegative fluid groups (Table 2.2).

Gomez and Thurston [55] investigated the strain-dependence of the elastic and viscous components of stress (τ'' and τ' respectively) of RA and DJD fluid. The strain dependence of τ' was similar for both fluids but τ'' showed a different response with fluid pathology [55]. For the DJD fluid, a yield stress was observed; τ'' then decreased and reached a plateau, while for the RA fluid no yield stress was observed, and τ'' reached a plateau gradually [55]. The level of τ'' was also lower for the RA fluid than the DJD fluid [55]. The authors attributed the difference of the behaviour of the elastic component of shear stress to a higher degree of elasticity found in the DJD fluid as opposed to the RA fluid; a looser structure with less ability to store energy is found in RA fluids [55].

Finally, Lai *et al*. [107] computed the relaxation spectra and stress relaxation functions from experimental data of the dynamic moduli of healthy and pathological synovial fluids. A significantly shorter relaxation time was obtained for osteoarthritic fluid than for normal synovial fluid. For normal synovial fluid, the relaxation time was in the order of 10 s, while for osteoarthritic synovial fluid it was in the order of 0.1 s. These authors developed constitutive equations to describe the rheological properties of synovial fluid [33] .

2.2.3 Effect of chemical composition on the viscoelastic properties of pathological synovial fluids

Several studies have attempted to relate the rheological properties of normal and pathological synovial fluids to their composition. It was identified early on that the flow properties of synovial fluid depend on the concentration of the HA protein complex [108]. Specifically, the apparent viscosity of synovial fluid at a shear rate of 1 sec^{-1} correlated well with HA/protein concentration [108]. It was not obvious however for early researchers whether the observed reduced viscosity of RA synovial fluid was due to the low concentration of HA or due to its lower molecular weight [91]. It has been postulated that high concentration and molecular weight of HA in synovial fluid are both essential for lubrication, damping action, and load bearing of joints [72]. The rheological properties of pathological synovial fluid are altered due to the presence of HA at both low concentration and low molecular weight [109,110].

HA concentration

The viscoelasticity of synovial fluid is a function of HA concentration; synovial fluid is elastic at high HA concentration [111] and higher concentrations of HA in normal, osteoarthritis and RA synovial fluids correlated well with rheological measurements [112,113]. Anadere *et al*. [89] found that the higher HA concentration found in synovial fluid from patients with meniscus defects and osteoarthritis as opposed to that in synovial fluid from patients with RA seronegative matched the greater elasticity manifested in the former fluids. A good correlation was found between the viscosity of synovial fluid from

patients with inflammatory and non-inflammatory joint diseases and HA concentration [51]. The median value of HA concentration in the non-inflammatory synovial fluid groups was found to be 0.720 mg/mL as opposed to 0.222 mg/mL from the low viscosity inflammatory groups.

The concentration of HA in normal, osteoarthritic and RA synovial fluid as obtained from various studies, is given in Table 2.1. The variability obtained in the ranges of concentration could be due to the difference in the chemical techniques used and also to patient-to-patient variability. However, for studies by Decker *et al.* [52] , where the same method of concentration determination was used for the three groups, the ranges indicate a clear transition from high to low HA concentration in normal, osteoarthritic, and RA fluids respectively.

HA molecular weight

Earlier studies on pathological synovial fluids indicated that HA is degraded in RA synovial fluid [114]. Subsequent studies by Bollet [54] also suggested a decrease in the degree of polymerization of HA of pathological synovial fluids of patients with RA and osteoarthritis. This decrease is reflected in the low molecular weights obtained for pathological synovial fluids compared to normal fluids in studies by Altmann *et al*. [115]. The molecular weight of HA in normal synovial fluid (7×10^6 Da) is considerably higher than that of RA synovial fluid (4.8×10^6 Da) [50]. The ranges obtained for normal and

RA patients from Dahl *et al*. [50] in Table 2.1 confirm this distinct difference. Studies by Schurz and Ribitsch [88] found that in normal synovial fluid the molecular weight of HA is 10^7 Da , while in rheumatoid and osteoarthritic synovial fluid it is $\leq 10^6$ Da and this could explain the deterioration of rheological properties in diseased fluids [88]. In this study however, synovial fluid dilution by effusion was not investigated which could also result in the degeneration of rheological properties [88].

Comparing RA to osteoarthritic synovial fluid, Ferguson *et al*. [116] found that concentration of RA synovial fluid by evaporation led to an increase in viscosity, but elastic properties were not restored. However, treatment of osteoarthritic synovial fluid with hydrogen bond breaking agents resulted in a decrease of viscosity and elasticity. More Newtonian behaviour was observed and relaxation times were considerably lowered. The authors attributed this behaviour to the presence of a macromolecular complex in osteoarthritic synovial fluid whose dissociation along with dilution results in the deterioration of viscoelastic properties in RA synovial fluid [116].

Both HA concentration and molecular weight are lower in diseased fluids. According to Stafford *et al*. [117], the low viscosity exhibited by synovial fluid from patients with rheumatic diseases could be due to both the depolymerization of HA as well as the decrease in its concentration. In these diseased states, decreased HA concentration could

arise from synovial fluid dilution and depolymerization could result from the presence of degrading components found in blood serum [117,118].

2.3 Periprosthetic fluid

2.3.1 Composition

Total knee arthroplasty (TKA) is a surgical procedure used to replace joints with osteoarthritis, rheumatoid arthritis and severe trauma [7]. Periprosthetic fluid is the natural lubricant generated *in vivo* after joint prosthesis surgery [120], yet few studies have investigated its properties. Saari *et al*. [121] studied the composition of joint fluid from patients with hip osteoarthritis at primary (before initial joint replacement surgery) hip arthroplasty and fluid from patients at revision THR (total hip replacement). Comparable protein concentrations were found in both groups, and similarly, protein and phospholipid concentration in osteoarthritic fluid at primary TKR (total knee replacement) were not statistically different from those at revision [122] (Table 2.3).

However, studies by Mazzucco *et al*. [8] indicated that whereas the protein content of fluid at primary TKA was 25% higher than that of revision TKA fluid, the phospholipid concentration did not differ between the two groups (Table 2.3).

Table 2.3 Composition of fluid at primary joint replacement and of fluid at revision joint replacement from various reports.

	Primary		**Revision**	
	Value	Ref.	Value	Ref.
HA $\overline{M}_w$ (MDa)	1.12 ± 0.84	[121]	2.63 ± 1.13	[121]
	1.8 ± 0.1	[8]	1.7 ± 0.2	[8]
			3.09 ± 0.883	[123]
HA (mg/mL)	2.21 ± 0.23	[121]	0.43 ± 0.04	[121]
	1.3 ± 0.5	[8]	0.9 ± 0.4	[8]
			0.64 ± 0.42	[123]
Protein (mg/mL)	24.8 ± 7.2	[122]	28.0 ± 5.0	[122]
	31.8 ± 1.5	[121]	34.4 ± 1.9	[121]
	27 ± 10	[8]	34 ± 13	[8]
Phospholipid (mg/mL)	0.4117 ± 0.1385	[122]	0.5028 ± 0.0589	[122]
	0.52 ± 0.18	[8]	0.52 ± 0.19	[8]

Although the concentration of HA in fluid at primary joint replacement was higher than that at revision [8,121], the molecular weight of HA at revision was either higher than at primary THR [121] or similar in both primary and revision TKA [8] (Table 2.3). Similar findings were observed in studies by Yamada *et al*. [123]; the concentration of HA in synovial fluid at revision THR was lower than that in osteoarthritic fluid while HA molecular weight determined by intrinsic viscosity did not vary significantly between the two groups. It should be noted that in studies by Mazzucco *et al*. [8], the difference in HA concentration between revision and primary TKA fluid was observed only for revision

cases due to prostheses wear related failure; for one case of fluid at revision for reasons unrelated to wear, the HA concentration was substantially higher than the mean value for fluids at revision due to wear related failure.

These studies suggested that after total joint replacement, a pseudo-synovial membrane [123] is formed and HA is synthesized and released into the prosthesis [121]. Moreover, the fluid that surrounds the prosthesis is similar to synovial fluid; it is a dialysate of blood plasma into which hyaluronate has been added [121].

2.3.2 Rheological properties

Fluids from patients undergoing TKA revision surgery were found to display non-Newtonian behaviour and shear-thinning [122,124]. The flow properties of fluids from patients undergoing total knee arthroplasty and revision arthroplasty were also determined by Mazzucco *et al*. [7]. The two groups differed in their shear rate dependence; fluids from patients undergoing TKA showed more shear-thinning [7]. Compared to normal synovial fluid, both groups displayed a decreased viscosity although the viscous parameters for fluid obtained at TKA were more similar to normal synovial fluid than those of fluid at revision TKA [7]; the viscous properties of fluid at revision TKA are degraded compared to fluid at TKA.

Compared to osteoarthritic fluid prior to TKA, periprosthetic fluid at TKA revision exhibits lower viscosity and decreased shear-thinning character at the shear rates tested

[122]. Studies on the dynamic moduli G' and G'' as a function of frequency for fluid at TKA and revision indicated that G'' was higher than G' at low frequencies, but above the cross-over frequency G' exceeded G'' [7]. Although the mean cross-over frequency was found to be lower in fluid prior to TKA than in fluid at revision TKA, this difference was not statistically significant. However, compared to normal synovial fluid, the cross-over frequency of both groups was higher.

2.4 Wear and friction of total knee arthroplasty

Mazzucco *et al*. [29] investigated the effect of joint fluid at primary and at revision knee replacement on the tribology of metal-on-polyethylene joint replacements. A varying amount of friction was produced with this system when lubricated with joint fluid. For certain individuals with total joint replacement, joint fluid is a poor lubricant for cobalt chromium alloy (Co-Cr) on polyethylene systems, and this can lead to a higher risk of wear. Lower friction was observed with oxidized zirconium-niobium alloy (Ox-Zr) as opposed to Co-Cr articulation on polyethylene. When compared with phosphate buffered saline alone, addition of HA and protein individually, increased the dynamic friction of Co-Cr on polyethylene articulation. The authors suggested that the boundary lubricant for metal on polyethylene articulation is a protein, or more proteins, and is unlikely to be lubricin.

Sawae *et al*. [125] investigated the effect of albumin and HA on the friction and wear of UHMWPE using cylindrical flat UHMWPE pins articulating against stainless steel and

alumina ceramic disc specimens. Albumin and sodium hyaluronate were added to saline and their effects on friction and wear were evaluated and compared to bovine serum. Less pronounced wear tracks were observed on the UHMWPE pin when articulated against alumina disc. Specifically, in sodium hyaluronate solution, almost all the ridges of machining marks remained on the UHMWPE surface. However, the worn surface of UHMWPE pin sliding against alumina in albumin solution showed a typical wear pattern of arrays of undulations arranged perpendicularly to the sliding direction. Under standard electron microscopy, almost no wear was observed on the UHMWPE surface sliding in sodium hyaluronate solution.

In saline and albumin solution, the friction between UHMWPE and prosthetic discs was higher than in diluted serum and HA solution. The lowest coefficient of friction and the lowest wear rate were observed when UHMWPE articulated against alumina in the presence of sodium hyaluronate solution. The authors postulated that hydrodynamic pressure due to the viscous nature of sodium hyaluronate could have been generated at the edges of the spiral ridges present on the initial surface of the pin [125]. It was suggested that the viscous nature of synovial fluid could reduce the friction and wear of joint replacements owing to the presence of HA [125].

DeJardins *et al.* [10] conducted knee simulator tests using a UHMWPE tibial bearing articulating against Co-Cr femoral bearing. Simulator tests were conducted at 37 ^{o}C for 5

million cycles using 50% bovine serum (BS) and 50% bovine serum with 1.5 g/l HA. Gravimetric analysis compared the wear rates of the UHMWPE tibial component at 0.5 million cycle intervals. Higher wear rate was observed in the BS with HA lubricant compared to the BS lubricant. Addition of HA to BS provided a clinically relevant lubricant since the flow properties of BS with HA lubricant was similar to joint fluid obtained at revision total knee arthroplasty. Simulator tested and retrieved Co-Cr femoral components revealed scratch marks parallel to the sliding direction.

Scholes *et al*. [126] investigated the effect of lubricant on the lubrication modes of metal-on-plastic, metal-on-metal and ceramic-on-ceramic joints. Full fluid film lubrication was observed with ceramic-on-ceramic joints using carboxymethyl cellulose as the lubricant. Metal-on-plastic and metal-on-metal hip joints operated under a mixed lubrication regime using this lubricant. Friction factors were two orders of magnitude lower for alumina-on-alumina joints than metal-on-metal bearings when lubricated with carboxymethyl cellulose. This was attributed to the hydrophilic nature of the ceramic components which enhanced their wetability and hence their lubrication properties. Under biological fluid lubrication, specifically bovine serum and synovial fluid, the friction factor increased in alumina/alumina (ceramic-on-ceramic) joints [127]. This was attributed to the adsorption of proteins forming solid like films on the surfaces of the joint, resulting in the breakdown of the fluid film in ceramic-on-ceramic joints. A solid-like film is adsorbed onto the surface and could be thick enough to break down part of the fluid film in

alumina/alumina joints [128]. For the metal-on-metal joints, the boundary lubricating ability of the adsorbed proteins led to a significant reduction of the coefficient of friction. Other studies also showed that the coefficient of friction increased with concentration of bovine serum in metal-on-plastic and in ceramic-on-ceramic joints, while the opposite was true for metal-on-metal joints [129]. Gispert *et al.* [130] also conducted pin-on-disc friction tests using several lubricants. Addition of hyaluronate decreased the coefficient of friction of salt solution. The authors argued for a hydrodynamic effect introduced to the saline solution by the high viscosity of HA.

The purpose of this thesis is to evaluate the friction properties of knee replacement components, and more specifically the role of joint fluid composition. This is accomplished by: 1) determination of the effect of fluid composition on its rheology, 2) establishing a method for friction determination in a reciprocal wear simulator, 3) investigation of the effect of fluid composition on friction in metal on UHMWPE, and 4) determination of the mechanism of friction behavior in these systems.

CHAPTER 3
EFFECT OF CONCENTRATION AND MOLECULAR WEIGHT ON THE RHEOLOGY OF HYALURONIC ACID/BOVINE CALF SERUM SOLUTIONS

3.1 Introduction

In simulator testing of knee and hip joints, it is necessary that the *in vitro* testing lubricant is representative of the *in vivo* joint fluid after arthroplasty as much as possible. Studies have been conducted in metal on polyethylene prosthetic joints to investigate the wear properties of prosthetic components in various lubricants containing phospholipids, soy protein, albumin, γ-Globulin [131-134]. These studies concluded that clinically relevant wear in polyethylene is only produced with serum lubrication [134]. *In vitro* wear testing for orthopaedic implants is currently conducted with BCS as the testing lubricant. However, although BCS may resemble periprosthetic fluid (the fluid that bathes total joint replacements *in vivo*) in protein composition, it does not contain HA [7,8]. Moreover, BCS is a Newtonian fluid while periprosthetic is a non-Newtonian fluid with observed shear thinning [7,8,10]. Therefore, recent studies have been conducted to incorporate HA in BCS in an attempt to create a more clinically relevant testing fluid [10]. Supplementing BCS with HA resulted in viscosity increase and shear thinning behaviour.

The objective of this chapter was to present a rigorous characterization of the rheological response of HA/BCS solutions in steady and oscillatory shear, in an effort to obtain a thorough understanding of the effect of HA addition on the rheology of BCS. Different concentrations of HA within physiological ranges were used to create test fluids, and rheological measurements were conducted to characterize these test fluids. The rheological behaviour of the HA solutions was compared with data that had been published for *in vivo* synovial fluids.

3.2 Materials and methods

3.2.1 Solutions

BCS was first prepared by the following additions to 500 mL stock BCS (HyClone™ Laboratories, Logan, UT): 20 mL of 0.2 micron filtered 15.4% EDTA solution (VWR Canlab™, Mississauga, ON) as a chelating agent to calcium, 5 mL of hydrated Fungizone and 3 mL of Penicillin Streptomycin (Invitrogen™ Canada Inc., Burlington, ON) as antimicrobial agents. BCS with protein concentration of 34 mg/mL was obtained (since this concentration was found in fluid at TKA revision arthroplasty [8]) by dilution with 0.2 micron filtered D.I. water. Filtration was conducted using sterile polyethersulfone filters (Nalgene®; Thermo Fisher Scientific, Rochester, NY).

Two molecular weights of HA were used to make HA/BCS solutions. The first, HA1 (M_w = 2.48 MDa), (Genzyme™, Cambridge, MA) was used to make HA/BCS solutions with HA concentrations of 0.6, 0.8, 1, 2, 3, 4 and 5 mg/mL. The second molecular

weight, HA2 (M_w= 1.0 MDa), (Fisher Scientific™, Whitby, ON) was used to make HA/BCS solutions with HA concentrations of 1 and 4 mg/mL.

3.2.2 Methods

Steady shear viscosity measurements

Steady shear rheology evaluations were conducted with a TA Instruments AR 2000™ rheometer (TA Instruments, New Castle, DE) equipped with a cone-and-plate fixture consisting of a 2^o, 6 cm diameter stainless steel cone, in the steady-shear mode (Figure 3.1). This fixture was most suited for conducting measurements for low viscosity fluids. A 2mL fluid sample was required and the temperature was controlled at 37^o C. For solutions containing low concentration of HA1(0.6 and 0.8 mg/mL) measurements were conducted with an ATS RheoSystems® Viscotech® rheometer (ATS RheoSystems, Bordentown, NJ), due to its better torque resolution. A 1.5 mL fluid sample was required and the temperature was also controlled at 37^o C.

Figure 3.1 TA Instruments AR 2000^{TM} rheometer.

Rheological measurements were conducted for three trial aliquots of each HA/BCS solution. The variation between trials was less than 8% based on the standard deviation at each viscosity measurement. It is important to note that in hip replacement and possibly knee replacement, the shear rates are well over 10^5 s^{-1} [12]. The measurements in this study involved shear rates of up to 10^3 s^{-1}. This is an inherent limitation in conventional rheometry, where equipment limitations and issues related to inertia during the measurement preclude measurements at higher shear rates.

Modeling of steady-shear viscosity data

The quantitative interpretation of the steady shear data from experimental tests used the modified Cross model that has been used previously in studies of joint fluids and periprosthetic fluid analogues [7,119]. It is a three parameter model of viscosity as a function of shear rate that takes into account the lower Newtonian plateau observed at low shear rates, as well as shear thinning character [135]. At the lower Newtonian plateau, the viscosity is constant at low shear rates. Shear thining occurs when the viscosity decreases with shear rate. The model is expressed by:

$$\eta = \frac{\eta_o}{1 + \left|\lambda \dot{\gamma}\right|^{(1-n)}} \tag{3.1}$$

where η is the viscosity, $\dot{\gamma}$ is the shear rate, η_o is the zero shear rate viscosity, and λ is the relaxation time. The flow index *n*, reveals the extent of shear thinning character where lower values of *n* indicate a more shear thinning material. The value of $\frac{\eta_o}{\eta_{\dot{\gamma}=300}}$ is the ratio of the zero shear rate viscosity to the viscosity at a shear rate of 300 s^{-1}. This is used as an additional measure of the extent of shear thinning character [4].

Non-linear regression analysis using the statistical software JMP^{TM} (Cary, NC, US) was performed to estimate the modified Cross model parameters. JMP^{TM} minimizes the residual sum of squares, performs the iterations until convergence is achieved and

provides the parameter estimates as well as their standard errors. For each HA/BCS solution, the data for all three replicate trials were used to obtain the model fit.

Viscoelastic measurements

In addition to steady shear properties, dynamic oscillatory rheological measurements were performed with a TA Instruments AR 2000^{TM} rheometer (TA Instruments, New Castle, DE) equipped with a 0.5^{o}, 6 cm diameter acrylic cone on plate fixture to determine the viscoelastic properties of HA/BCS solutions. Temperature was controlled at 37^{o}C and a 0.5 mL fluid sample was required for each measurement. The limit of linear viscoelasticity, where rheological properties are strain independent, was determined by strain sweep experiments. The onset of nonlinearity occurred at 10% strain for all the solutions studied.

The viscoelastic properties were evaluated using frequency sweep measurements at 1-3% strain, which was well within the linear viscoelastic region. Within the linear viscoelastic region, the structure of the sample is maintained and the rheological response is independent of the magnitude of the deformation. Measurements were conducted for three trial aliquots of each HA/BCS solution and the average values of the shear storage (elastic) moduli (G'), shear loss (viscous) moduli (G''), and complex viscosity (η^*) were obtained as functions of the frequency, . The variation between the trials of the dynamic moduli and the complex viscosity was less than 15%.

It should be noted that oscillatory measurements are only reported for HA1/BCS solutions of concentrations above 3 mg/mL, given that at lower concentrations or molecular weights the signals were very noisy due to torque limitations of the rheometer.

3.3 Results

3.3.1 Steady shear rheology

The steady shear rheological properties of HA/BCS solutions of varying concentrations and molecular weights are shown in Figure 3.2 and Figure 3.3. At low shear rates, where molecules are highly entangled and most resistant to flow, high viscosity is observed. At higher shear rates, shear thinning occurs, where the viscosity decreases with shear rate resulting from molecular disentanglement and alignment in the shear field.

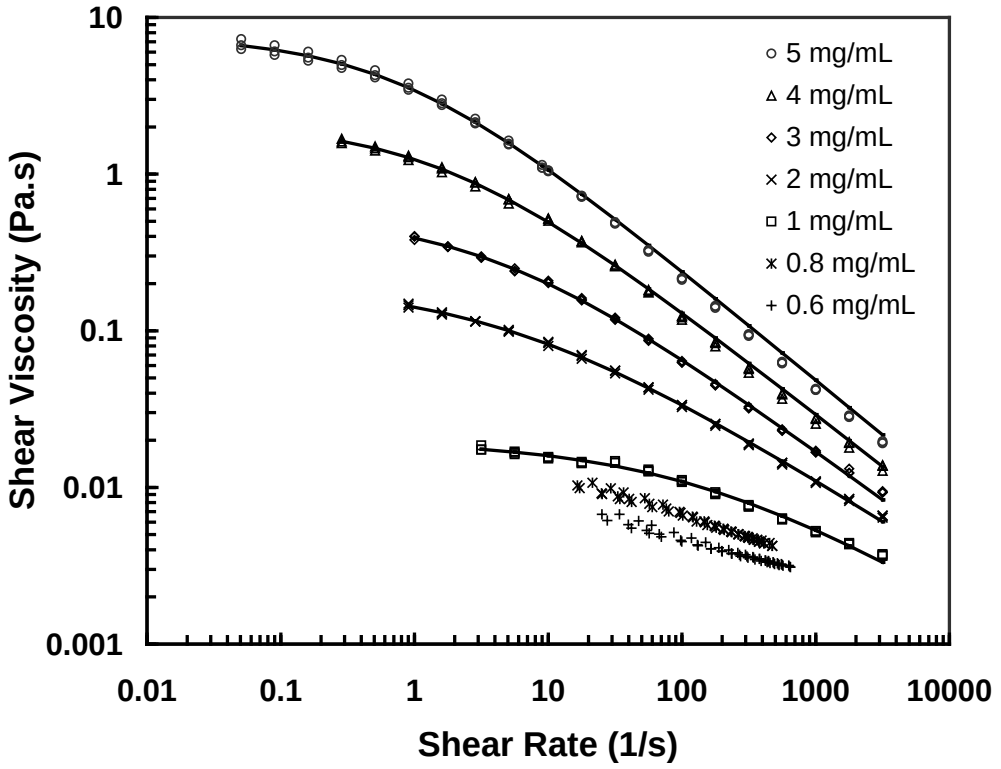

Figure 3.2 Viscosity vs. shear rate data for HA1/BCS solutions containing different concentrations of HA1, at 37°C. For each solution, measurements for the three trial aliquots are plotted. Solid lines indicate the modified Cross model fits. Note that there are no modified Cross model fits for low concentrations of HA.

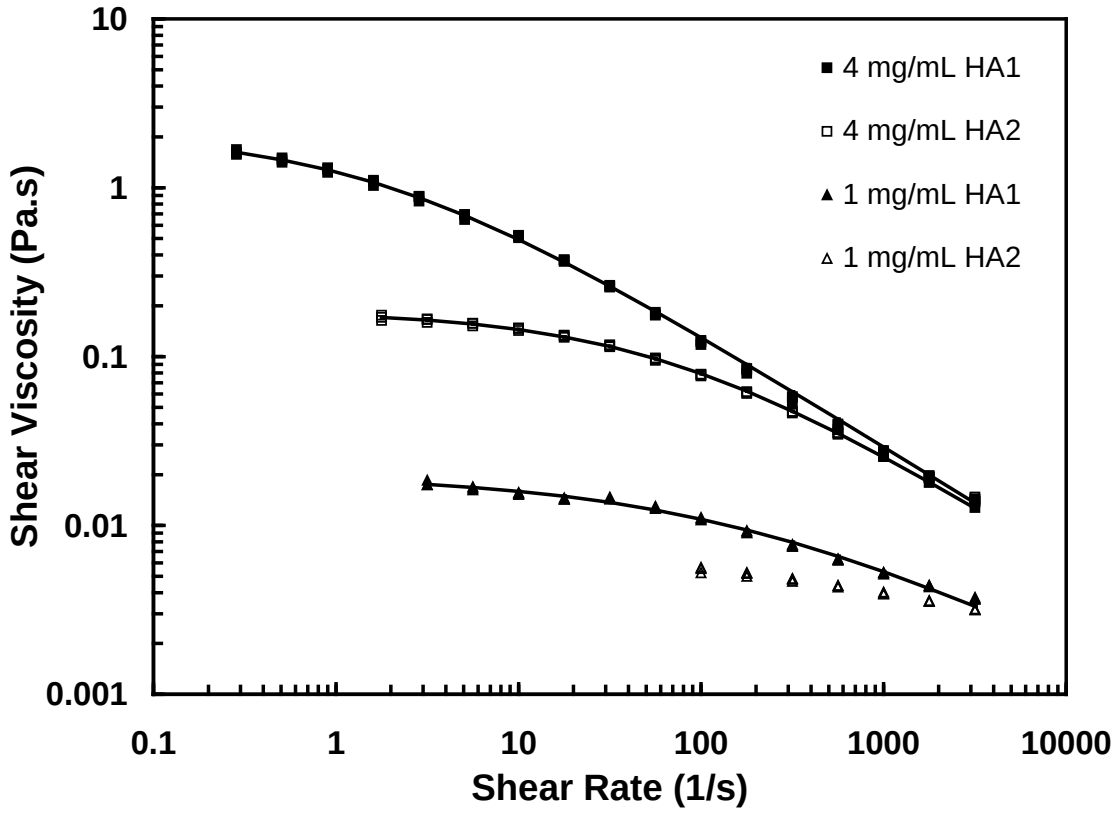

Figure 3.3 Viscosity vs. shear rate data for HA1/BCS and HA2/BCS solutions at 1mg/mL and 4 mg/mL HA at 37°C. For each solution, measurements for the three trial aliquots are plotted. Solid lines indicate the modified Cross model fits.

The modified Cross model fits are also shown in Figure 3.2 and Figure 3.3 and all parameters are summarized in Table 3.1. Hypothesis tests indicated that all the modified Cross model parameters were significant at the 95% level and that the model was adequate in predicting the rheological behaviour of these solutions. There was no significant model lack of fit for the viscosity vs. shear rate data of 2, 3, 4 and 5 mg/mL HA/BCS solutions. However, for solutions below 1 mg/mL, there was a significant lack

of fit, due to the absence of a well-defined Newtonian plateau. Therefore Cross model parameters are not reported for solutions below 1 mg/mL. For these solutions, the highest viscosity which was also obtained at the lowest shear rate is recorded in Table 3.1.

Table 3.1 Modified Cross model estimates for each parameter for HA/BCS solutions at 37°C.

Solution	η_o **(Pa.s)**	λ_r **(s)**	**n**	$\frac{\eta_o}{\eta_{\dot{\gamma}=300}}$
(0.6 mg/mL) HA1	0.007*	-	-	-
(0.8 mg/mL) HA1	0.011*	-	-	-
(1mg/mL)	0.020	0.007	0.478	2.477
HA1	(0.019-0.021)	(0.005-0.009)	(0.429-0.526)	
(2mg/mL)	0.200	0.196	0.460	10.002
HA1	(0.192-0.209)	(0.165-0.238)	(0.441-0.479)	
(3mg/mL)	0.558	0.256	0.375	16.112
HA1	(0.534-0.585)	(0.219-0.304)	(0.352-0.396)	
(4mg/mL)	2.114	0.592	0.331	32.997
HA1	(2.025-2.216)	(0.508-0.700)	(0.302-0.359)	
(5mg/mL)	7.630	1.341	0.298	68.334
HA1	(7.260-8.074)	(1.109-1.671)	(0.246-0.347)	
(4mg/mL)	0.186	0.016	0.328	3.817
HA2	(0.182-0.190)	(0.014-0.017)	(0.301-0.353)	

* This is the highest viscosity obtained and not η_o **,** since data for these solutions could not be fit to the modified Cross model. Brackets indicate the 95% confidence interval.

With increased HA concentration in the BCS solution, the viscosity and the extent of shear thinning increases (Figure 3.2 and Figure 3.3). This is also reflected by the

corresponding values of the zero shear rate viscosity and the flow index, n, determined by the Cross model, as well as the calculated values of $\frac{\eta_o}{\eta_{\dot{\gamma}=300}}$ which are shown in Table 3.1.

Similar rheological behaviour has been obtained with the increase in HA concentration in saline and in a phosphate buffered solution [60,74]. With increased concentration in solution, a transient entanglement network arises where HA molecules entangle and disentangle after a period of time [73,74]. This network is stabilized by intermolecular associations of hydrogen bonding. As the concentration of HA increases in solution, the presence of a network gives rise to enhanced viscosity and pronounced extent of shear thinning [60,74,76]. This effect becomes more prominent at concentrations of HA above 4 mg/mL, which exhibit a marked increase in zero shear rate viscosity, as well as higher relaxation times, λ_r, which are indicative of the time needed for chains to disentangle (Table 3.1).

As expected, the rheology of the HA/BCS solutions is also strongly influenced by the molecular weight of HA, as shown in Figure 3.3 and the corresponding Cross model parameters in Table 3.1. Solutions containing HA of lower molecular weight exhibit significantly lower viscosity. Additionally, the onset of non-Newtonian behaviour takes place at higher shear rates, resulting in lower relaxation times. In HA1/BCS solutions the longer chains contained in the higher molecular weight HA allow for a greater number of entanglements, leading to increased viscosity and enhanced shear thinning character in

these solutions. Similar trends were obtained for HA in water by Ambrosio *et al*. [80]; the viscosity vs. shear rate curve of HA solutions with HA of molecular weight 0.15 MDa displayed Newtonian behaviour while the curve for the HA solution with molecular weight of 1.2 MDa displayed significant viscosity increase and strong shear thinning behaviour.

3.3.2 Viscoelastic properties

The frequency dependence of the dynamic moduli, G' and G'' is depicted in Figure 3.4 and Figure 3.5 respectively. The magnitudes of G' and G'' increase with higher concentration and molecular weight of HA in solution. The increasingly elastic character of the solutions upon increasing concentration and molecular weight is illustrated by plotting the loss tangent ($\tan\delta = \frac{G''}{G'}$) versus frequency (Figure 3.6).

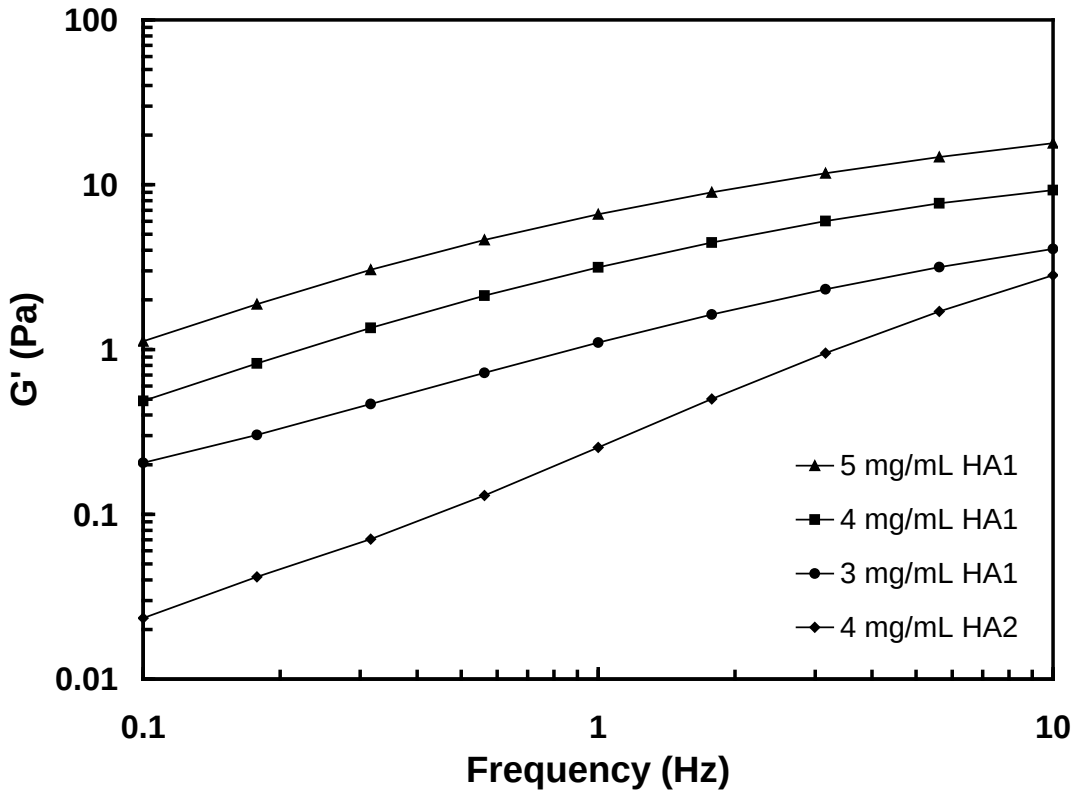

Figure 3.4 Viscoelastic properties of HA/BCS solutions as functions of frequency at 37°C, Storage moduli.

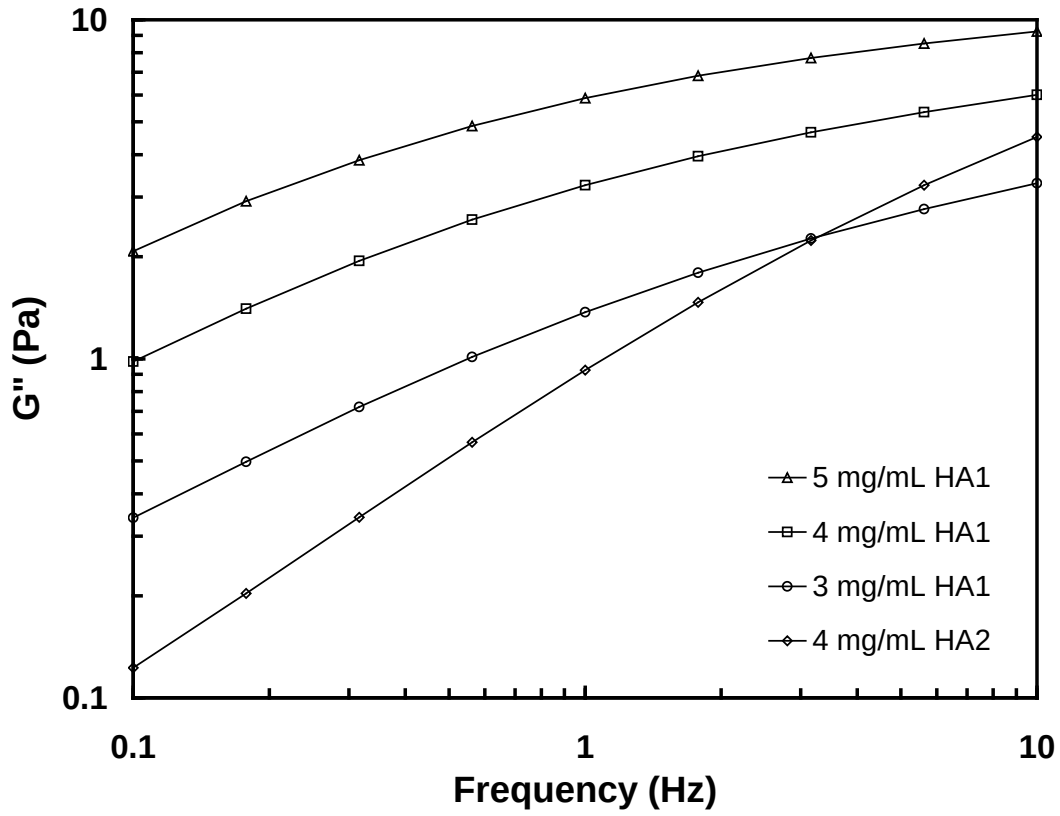

Figure 3.5 Viscoelastic properties of HA/BCS solutions as functions of frequency at 37°C, loss moduli.

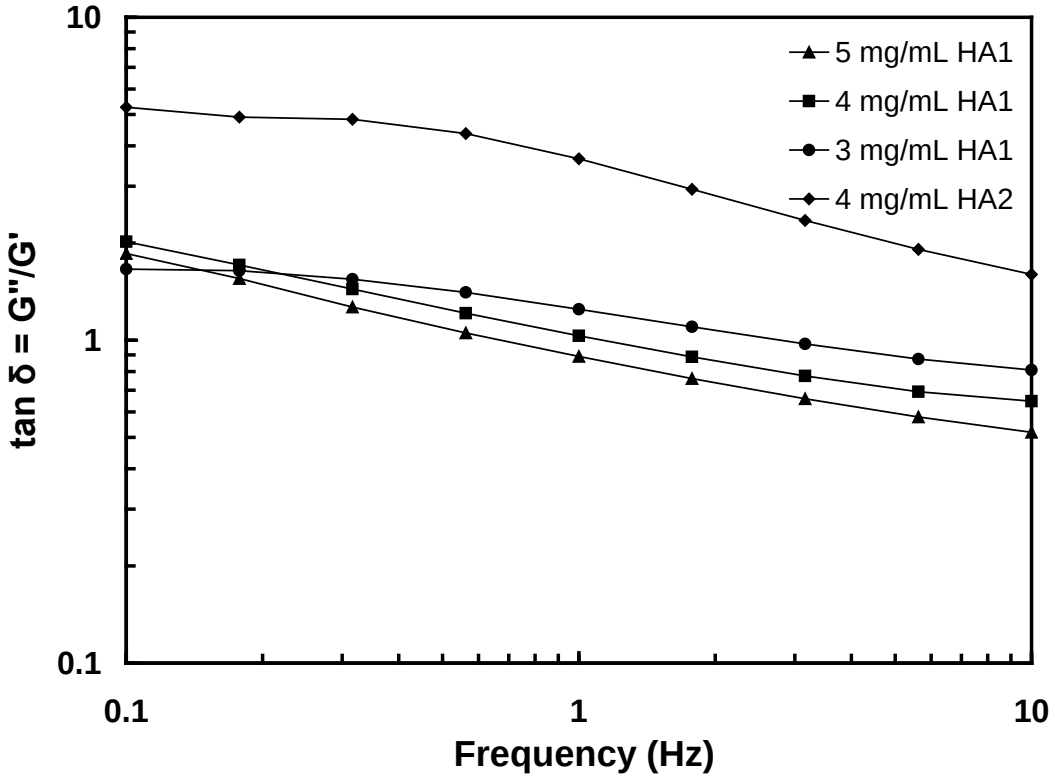

Figure 3.6 Viscoelastic properties of HA/BCS solutions as functions of frequency at 37°C, loss tangent (tanδ).

The cross-over moduli and corresponding frequencies, which correspond to the disentanglement rate of the polymer chains [90], are determined by combining the elastic and loss moduli as shown in Figure 3.7 and Figure 3.8.They are also summarized in Table 3.2. The disentanglement rate reflects the mobility of the polymer chains, hence the cross-over frequency typically depends on polymer concentration and molecular weight [80]. With increased HA concentration and molecular weight in solution, the disentanglement rate decreases, leading to a decrease in the cross-over frequency [80]. This is reflected in

the results shown in Table 3.2, where the cross-over frequency decreases with increasing HA concentration in solution. Similar results were obtained by De Smedt *et al*. [73] in phosphate buffered saline solutions containing HA.

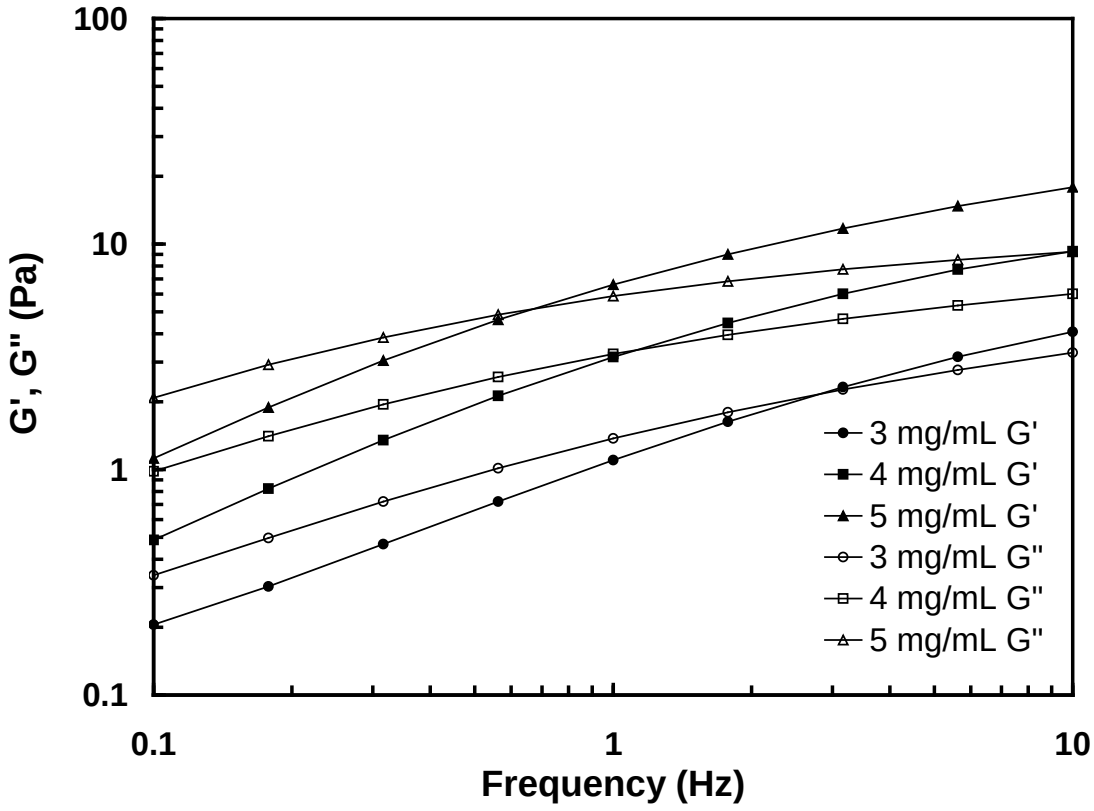

Figure 3.7 Dynamic moduli as a function of frequency at 37°C for HA1/BCS.

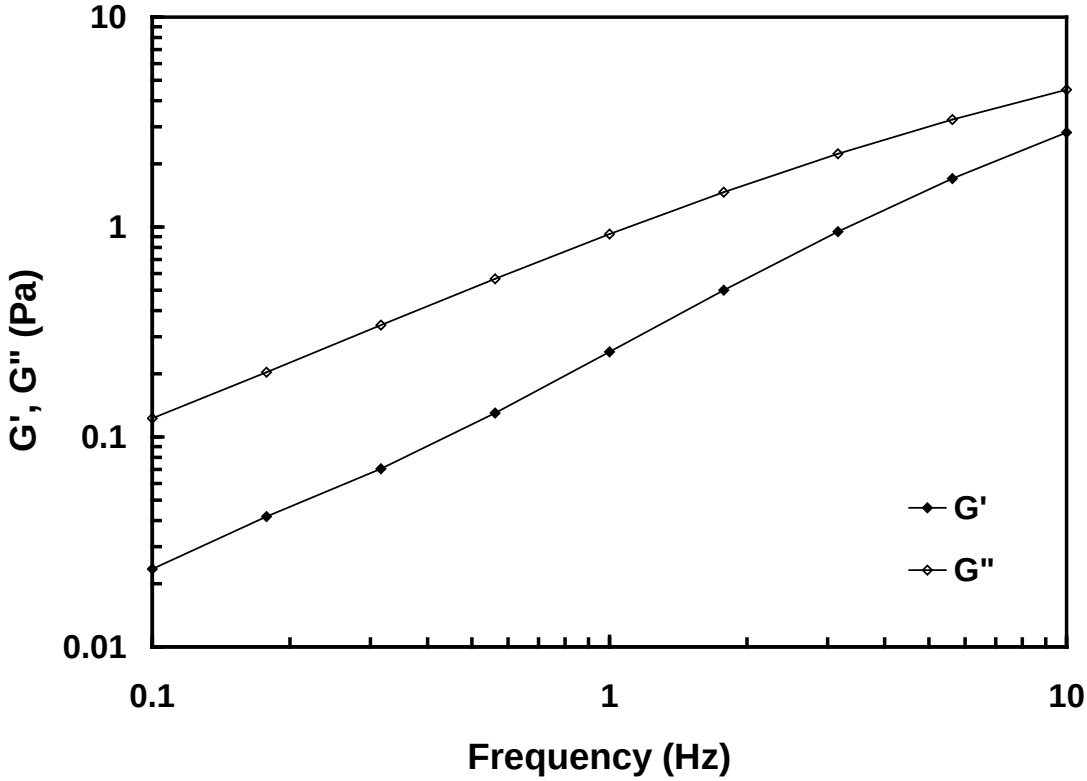

Figure 3.8 Dynamic moduli as a function of frequency at 37°C for HA2/BCS.

Table 3.2 Cross-over frequency for HA1/BCS solutions at 37°C.

	f_c **(Hz)**	G_c **(Pa)**
HA (3 mg/mL)	2.80	1.9
HA (4 mg/mL)	1.14	3.0
HA (5 mg/mL)	0.70	5.0

Figure 3.9 summarizes the complex viscosities of the solutions as a function of HA concentration. These superimpose well with the steady shear viscosities according to the Cox Merz rule which states that $|\eta^*(\omega)|$ is identical to $\eta(\dot{\gamma})$, where $\dot{\gamma} = \omega$ (in rad/s) [136].

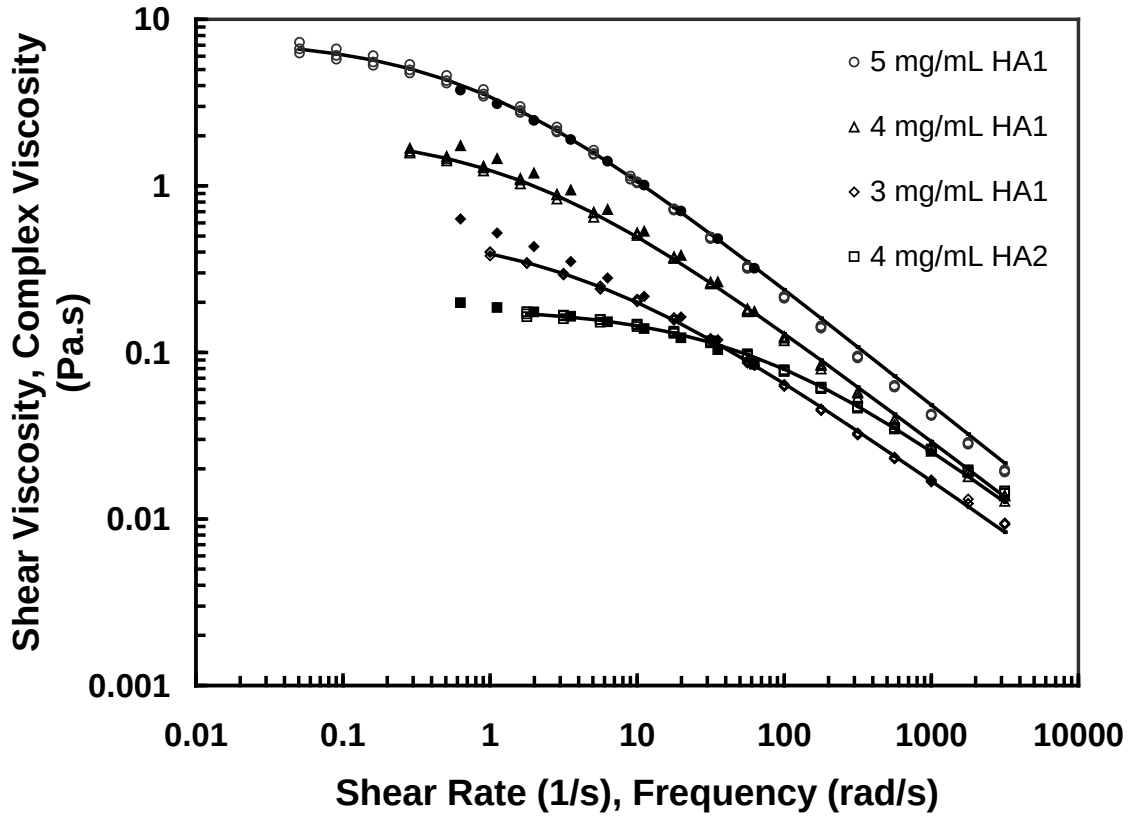

Figure 3.9 Viscosity as a function of shear rate and complex viscosity as a function of angular frequency for HA1/BCS and HA2/BCS solutions at 37°C. Open symbols indicate steady shear viscosity and closed symbols indicate complex viscosity measurements. Solid lines indicate the modified Cross model fits.

3.4 Discussion

Knowledge of the rheological properties of HA/BCS solutions used is important in order to establish a lubricating environment that is consistent with operating conditions *in vivo*. The transition from viscous to elastic behaviour is of particular interest, because it determines to what extent the fluid absorbs, or dissipates energy.

The cross-over behaviour is an inherent characteristic of synovial fluids of varying pathology; cross-over frequency decreases from rheumatoid arthritis to osteoarthritis to normal synovial fluids where HA concentration increases respectively [4]. No cross-over is observed in rheumatoid arthritis seropositive synovial fluids due to decreased HA molecular weight in these fluids [90].

In the solutions under consideration in this work, the cross-over frequency varies with respect to HA concentration, thus providing a range of cross-over frequencies, which can be related to those found in synovial fluids of varying pathology. The solutions containing the lower molecular weight, HA2 did not display cross-over in the frequency range investigated, making them potential analogues of rheumatoid arthritis seropositive synovial fluids. With increased concentration and molecular weight of HA in solution, elastic properties are enhanced as can be observed by the increase in their storage moduli (Figure 3.4), decrease in loss tangent (Figure 3.6), and the decrease in cross-over frequency (Table 3.2 and Figure 3.7). These properties may interact with other factors contributing to load bearing in joint function. For example, the potential of the fluid to deform under

load, combined with deformation of the bearing surfaces, is likely to influence the stress distribution in the load bearing region. However, the nature of this interaction is not known. According to Schurz [88], in normal synovial fluid, the viscoelastic properties imparted by both the concentration and molecular weight of HA are necessary for lubrication, damping action and load bearing and are required to prevent mechanical trauma and wear of articular cartilage during load bearing movement [88].

The steady shear rheological properties of the HA/BCS solutions can be also correlated with those of synovial fluids of different pathology. Based on the magnitudes of the zero-shear rate viscosity in Table 3.1, the 4 mg/mL and the 5 mg/mL HA1/BCS solutions are representative of the rheological properties of normal synovial fluid (Table 3.3). The rheological behaviour of the 2, and 3 mg/mL HA1/BCS solutions and the 4mg/mL HA2/BCS solutions is more characteristic of osteoarthritic synovial fluid (Table 3.3). Similar findings were also observed in studies by Aurora *et al*. [137] where a hyaluronate of similar concentration and molecular weight was used. The 1mg/mL HA1/BCS solution best represents inflammatory fluids such as rheumatoid arthritis (Table 3.3). The value of $\frac{\eta_o}{\eta_{\dot{\gamma}=300}}$ also reveals similar representative trends (Table 3.1, Table 3.3).

Table 3.3 Rheological parameters for normal, osteoarthritis and rheumatoid arthritis synovial fluid. Concentration and molecular weight of HA in these fluids are also included. Data from Schurz and Ribitsch [88] and from Rainer and Ribitsch [87] were obtained at 25°C.

	Normal		**Osteoarthritis**		**RA**	
	Value	Ref.	Value	Ref.	Value	Ref.
η_o (Pa.s)	6-175	[87]	0.1-1	[88]	0.004-0.07	[88]
	1-40	[88]				
$\frac{\eta_o}{\eta_{\dot{\gamma}=300}}$	70-250	[87]	5-40	[88]	1-4	[88]
	100	[88]				
HA (mg/mL)	2.50-3.65	[52]	1.07-2.60	[52]	0.39-2.19	[52]
	1.45-2.94	[53]	0.32-3.61	[55]	0.70-3.74	[55]
	2.0-4.0	[40]	1.24-2.22	[54]	0.37-1.88	[53]
	0.35-4.22	[54]			0.19-1.26	[50]
					0.35-2.41	[54]
					0.8-1.7	[56]
HA $\overline{M}_w$ (MDa)	6.3-7.6	[50]	1.06-3.48	[51]	3.2-6.8	[50]

3.5 Conclusions

The steady shear viscosity of HA/BCS solutions has been determined as a function of concentration and molecular weight. With increased concentration and molecular weight of HA in the BCS medium, higher viscosity and greater extent of shear thinning was observed. In the oscillatory mode of deformation, a pronounced increase in elastic properties and a decrease in cross-over frequency were observed upon increasing the concentration of HA and its molecular weight. The rheological behaviour HA/BCS

solutions of different concentration and molecular weight mimics that of synovial fluid of different joint pathology. However, the effect of the observed rheological properties on the friction properties of total knee arthroplasty is yet unknown.

CHAPTER 4
METHOD FOR FRICTION ESTIMATION IN RECIPROCATING WEAR TESTS

4.1 Introduction

It is recognized that friction measurements may reveal the mode of lubrication occurring within joint prostheses. Boundary and hydrodynamic fluid film lubrication are the mechanisms known to take place in joint replacements [12]. Hydrodynamic fluid film lubrication of bearing surfaces occurs when a viscous film is generated separating the two surfaces [138]. The two surfaces must move relative to each other with sufficient velocity for the hydrodynamic film to be generated [138]. If the hydrodynamic pressure is insufficient to separate the surfaces, then lubrication would primarily depend on the components of the fluid that could form boundary films [139]. In boundary lubrication, these components adsorb on the bearing surfaces and aid in minimizing friction upon contact. In mixed lubrication, the surfaces are partially separated by a fluid film, and partially in contact at the asperities [138]. Lubricant viscosity is a critical determinant in hydrodynamic fluid film lubrication, and hence it is important to consider its effect on the friction behaviour of joint prosthesis bearing surfaces [137]. This is particularly important since periprosthetic fluid, the fluid that bathes joint replacements *in vivo* contains HA that imparts it with viscous characteristics.

Friction studies have been conducted to determine the lubrication mechanism in which different bearing materials of joint replacements operate [126,127,129]. This is typically performed by using the Stribeck analysis where the friction factor is plotted against the Sommerfeld number [126,127]. A decrease in the friction factor with increase in Sommerfeld number is indicative of mixed lubrication while an increase in the friction factor with Sommerfeld number is indicative of fluid film lubrication [31,127,129]. Based on this analysis Flannery *et al*. [31] suggested that in total knee replacement a mixed lubrication regime dominates.

While simulator testing has focused on recreating the conditions of loading and motion of joints in service [17,19], friction measurements tend to use simpler test conditions at constant speeds [29]. This strategy makes it possible to delineate the factors influencing the lubrication mechanisms associated with the Stribeck curve. However, wear studies have shown a relationship between the nature of surface motion (rolling, sliding, cross-shear, etc.) and wear patterns [140]. Therefore, in order to study the role of lubrication in this phenomenon, it is necessary to measure friction under the dynamic conditions associated with joint replacements.

The purpose of this study was to determine a method for estimating the coefficient of friction in a linear reciprocating machine under kinematic and loading conditions representative of those encountered in total knee replacement. A reciprocating pin on disc

friction and wear testing machine (AMTI Ortho-Pod^{TM}, AMTI^{TM}, Watertown, MA) was used with D.I. water lubrication. A protocol for data acquisition and analysis was designed, followed by statistical analysis that quantified the variability of the coefficient of friction among different stations and tests. This study was designed to be used as basis for future work involving different lubricants, test geometries, and surface kinematics.

4.2 Methods and Materials

4.2.1 Instrumentation

An AMTI Ortho-Pod^{TM} six station pin on disc friction and wear testing machine was used in this study (Figure 4.1). Different motion paths can be generated by rotations of the pin and disc. There are two assemblies involved in this setup, the top head and the base. The top head contains the CoCr pin actuators while the base contains the discs where the UHMWPE specimens are mounted. The pin is loaded by air pressure and rotates with either reciprocating or rotary motion. A total of six individual fluid channels each enclose a pin and disc. A water bath is used to heat the unit using heat transfer passages in the base plate and upper head assembly. A computer controls the machine to the desired disc rotation, pin rotation and vertical load.

Figure 4.1 The AMTI Ortho-Pod^{TM} six station pin on disc friction and wear testing machine.

4.2.2 Test conditions

A 25 mm radius hemispherical CoCr metal pin was articulated against a flat UHMWPE sample (Figure 4.2). This arrangement represents the non-conforming geometry articulation typical of a total knee replacement [16]. For each test, a load of 210 N was applied, resulting in a nominal average contact stress of 20-30 MPa representative of that encountered in total knee replacement [140]. The CoCr pin articulated against the UHMWPE sample in a unidirectional reciprocal sliding pattern, as shown in Figure 4.2 at a frequency of 1Hz. A straight line pattern was obtained by programming the rotations of the pin and disc to track seven points in both the forward and backward directions,

yielding a path length of 26 mm and a corresponding average speed of 26mm/s. Motion starts at Point A in the path depicted in Figure 4.2, continues to Point B where the pin stops and returns to Point A. D.I. water was used as the lubricant in each of the channels. The temperature of each of the channels was controlled using a water bath at 37 ^{o}C.

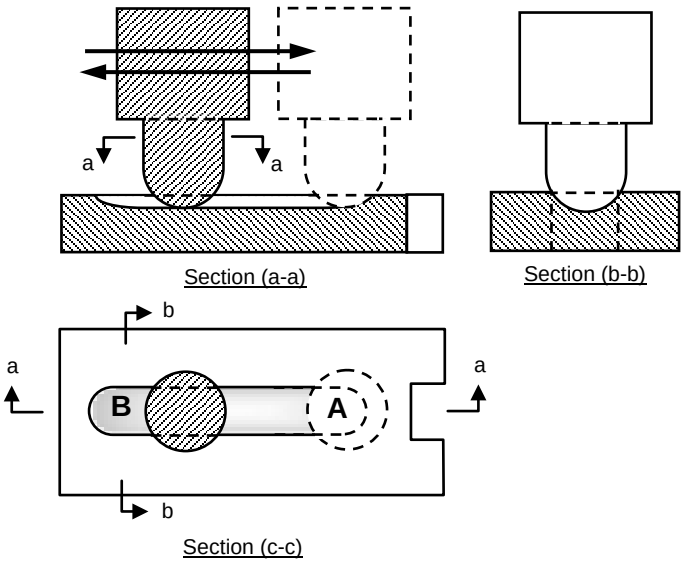

Figure 4.2 CoCr Pin and UHMWPE specimen arrangement and motion. Arrows indicate the reciprocal motion in a straight line path of the pin against the sample. Sections a-a, b-b, and c-c are side, front, and top views respectively of the pin articulating against the sample. Points A and B are the stop points in the path. The shaded pin is in an intermediate position and the dashed outlined pin is in the stop position.

4.3 Experimental protocol

4.3.1 Pin Polishing

Three tests were conducted for each experimental condition using three different channels. The CoCr pins were polished prior to each test to a mirror finish using a protocol typical for commercial implant components. Three different slurries were used in polishing the pins: a 6-micron diamond spray, followed by a 0.05-micron alumina slurry, then followed by a colloidal silica solution. Each slurry was sprayed on cotton cloth-wrapped rotating discs, and the pins were polished against the rotating discs for three to five minutes. Following each polishing, the pins were washed with soap water, rinsed with water and air dried. The surface of each pin was then examined using a light microscope with 40X magnification. Details of this procedure are given in Appendix B, Section B.3.

4.3.2 Sample Preparation

The samples were cut from an UHMWPE sheet (McMaster Carr, Aurora, OH). They were first rinsed with tap water to remove bulk contaminants and washed in an ultrasonic cleaner in a solution of 1% ionic detergent ((Liqui-Nox®) Alconox Inc., White Plains, NY) for fifteen minutes. They were then rinsed with a stream of distilled water and placed in the ultrasonic cleaner in a beaker of distilled water for five minutes to remove the detergent. The samples were dried with lint-free tissue and were left to air dry. Each test was conducted with a polished pin and a new UHMWPE sample.

4.4 Data Acquisition

The data acquisition system of the AMTI Ortho-Pod™ collects multi-component force data and the coefficient of friction. Force sensors in each of the three vertical legs of the machine measure three force components. The nine outputs are summed to provide a single value for each of the forces F_x, F_y, and F_z where x and y are the transverse direction and z is the normal direction. The coefficient of friction is determined by:

$$\mu = \frac{F_f}{F_z} = \frac{\sqrt{(F_x)^2 + (F_y)^2}}{F_z} \tag{4.1}$$

Each test was programmed with ten loops of 10,000 repetitions for a total of 100,000 cycles. The sampling rate was 400 data sets/second for a duration of ten seconds. Data were collected for the last ten cycles of the first 10,000 cycle loop. Additionally, data were collected for the last ten cycles at the end of the final loop.

4.5 Data Analysis

The vertical load and the friction force for two cycles are plotted in Figure 4.3. Periodic behavior is observed every second which corresponds to the cycle frequency of 1Hz. The coefficient of friction output for the two cycles is depicted in Figure 4.4 showing periodic behavior every 400 data points. Maxima and minima were observed every 200 data points corresponding to the stop points (Points A and B in Figure 4.2). As the pin approaches a stop point, the horizontal forces (F_x and F_y) increase as contact takes place

at the end of the path, resulting in an artificially high coefficient of friction. As the motion reverses, the horizontal forces approach zero as the pin stops, yielding an artificially low coefficient of friction.

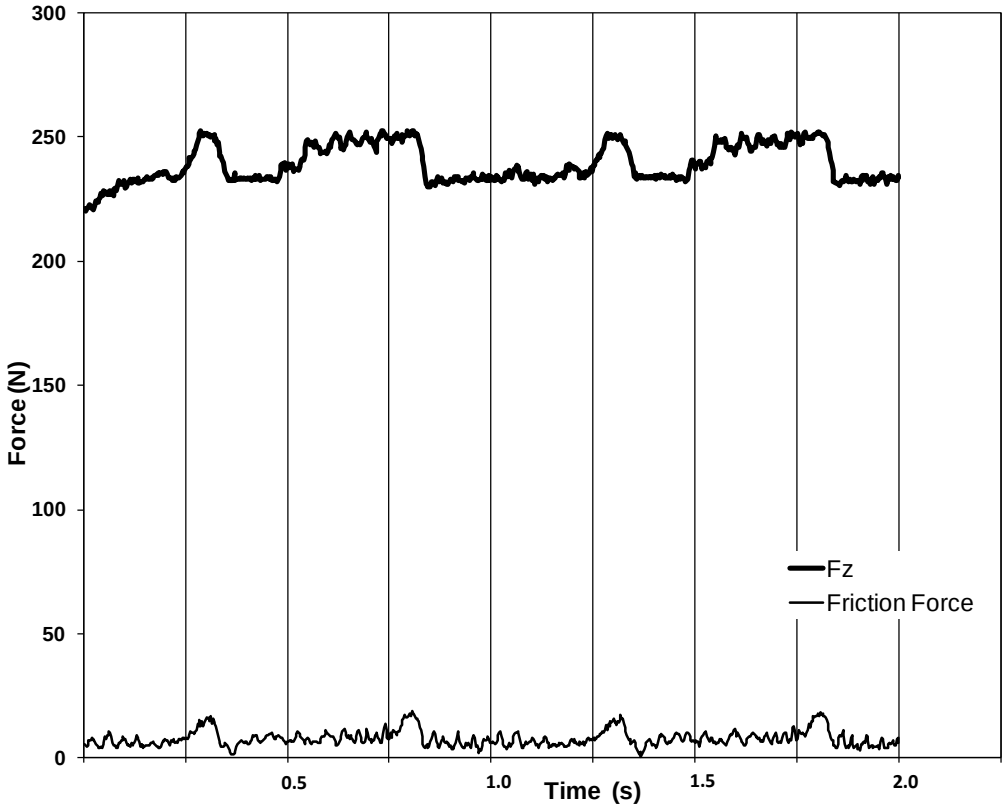

Figure 4.3 Vertical load and friction force vs. time.

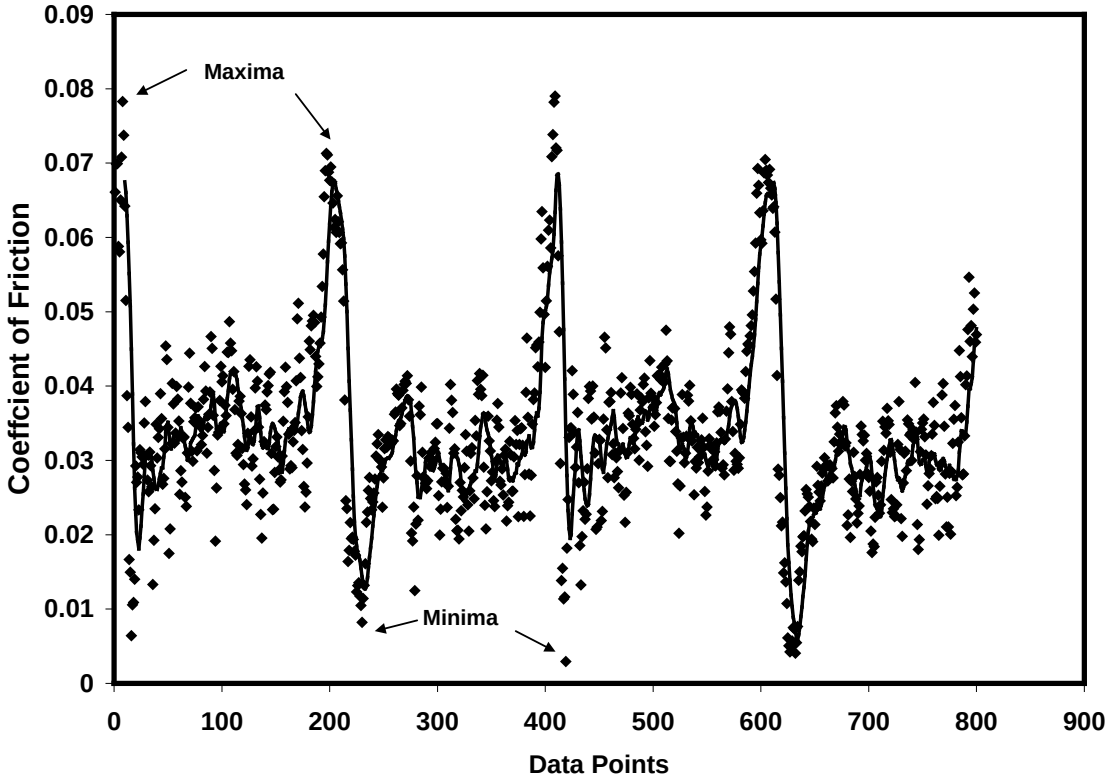

Figure 4.4 Typical AMTI Ortho-Pod^{TM} coefficient of friction output. In this plot, the coefficient of friction was plotted for two cycles under lubrication with D.I. water. The solid line indicates the moving average of 10 points. Maxima and minima are also indicated.

The group of data points between the minima corresponds to data obtained along the straight line path between Point A and Point B in Figure 4.2. It should be noted that the output from the three stations was slightly offset and data were synchronized by aligning the maximum points of the three outputs.

The number of data points required to estimate the coefficient of friction in each cycle was first determined. The standard error of the mean of 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 coefficient of friction data points about the midpoint of the cycle was computed.

The number of cycles required to compute the average coefficient of friction was also determined. The average coefficient of friction was computed for seven cycles and cycle to cycle variation was determined for each station and for three consecutive tests. The averages of the first 3, 4, 5, 6 and 7 cycles were determined and compared.

4.6 Results and Discussion

In Figure 4.5, the standard error of the mean of 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 coefficient of friction data points about the midpoint for the first cycle is plotted for the three stations used in the test. As expected, the variability of the calculation of the coefficient of friction decreased as the number of points increased. However, an asymptotic value of the standard error of the mean was observed at 30-35 points. As such, it was determined that using 30 data points about the midpoint was sufficient for computation of the coefficient of friction.

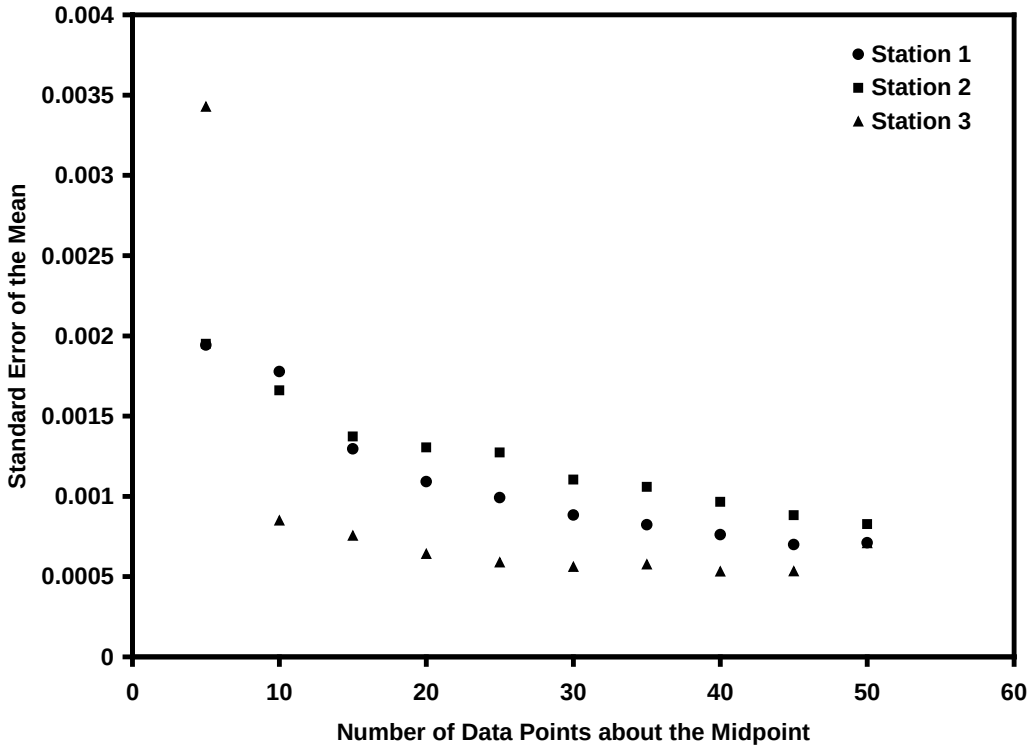

Figure 4.5 Standard error of the mean vs. number of data points about the midpoint.

To determine cycle to cycle variation, the average of 30 data points of the first 3, 4, 5, 6 and 7 cycles was determined. These averages were almost identical with a pooled standard deviation of 0.00026. Furthermore, this standard deviation was one order of magnitude lower than those obtained from test to test and from station to station. It was concluded that computing the average of the first three cycles was sufficient.

An additional observation from many tests was the presence of two groups of data with slightly different average values of the coefficient of friction. This is illustrated in Figure

4.4 in which the midpoint averages appear to alternate between higher and lower values. Using the 30-point and 3-cycle criteria, the coefficients of friction were computed after 10,000 cycles for three stations and three tests as summarized in Table 4.1 and Table 4.2 for the slightly higher and lower value groups respectively. The standard deviations obtained from station to station and from test to test are also indicated. For the higher value group, the overall mean of the coefficient of friction was 0.0381. The pooled standard deviation between stations is 0.0048 and that obtained between tests is 0.0046. Corresponding values for the lower value groups are shown in Table 4.2.

An unpaired student's t-test was conducted and the difference between the average coefficient of friction obtained from the lower and the higher value groups was not statistically significant at the 5% level. Therefore, in the subsequent analysis only average values of all groups are reported.

Table 4.1 The coefficient of friction and average standard deviation obtained for the three tests using three stations after 10,000 cycles for higher value groups.

Test	**Station 1**	**Station 2**	**Station 3**	**Station AVG**	**Station SD**
1	0.0318	0.0497	0.0413	0.0409	0.0090
2	0.0345	0.0410	0.0369	0.0375	0.0033
3	0.0377	0.0335	0.0361	0.0358	0.0021
Test AVG	0.0347	0.0414	0.0381	0.0381	**0.0048**
Test SD	0.0030	0.0081	0.0028	**0.0046**	

Table 4.2 The coefficient of friction and average standard deviation obtained for the three tests using three stations after 10,000 cycles for lower value groups.

Test	Station 1	Station 2	Station 3	AVG	Station SD
1	0.0262	0.0433	0.0399	0.0365	0.0090
2	0.0337	0.0363	0.0366	0.0355	0.0016
3	0.0359	0.0291	0.0356	0.0335	0.0038
AVG	0.0319	0.0362	0.0374	0.0352	**0.0048**
Test SD	0.0051	0.0071	0.0023	**0.0048**	

In Table 4.3, the station to station and test to test variability after 100,000 cycles is shown. The overall mean of the coefficient of friction was 0.047. The pooled standard deviation between stations is 0.0035 and that obtained between tests is 0.0036. From the average standard deviation between stations and between tests, the standard deviation of 0.005 was determined to be the inherent standard deviation in testing to be used for comparison of coefficient of friction under different lubricating environments.

Table 4.3 The coefficient of friction and average standard deviation obtained for the three tests using three stations after 100,000 cycles.

Test	Station 1	Station 2	Station 3	AVG	Station SD
1	0.0456	0.0508	0.0431	0.0465	0.0039
2	0.0455	0.0494	0.0447	0.0465	0.0025
3	0.0511	0.0441	0.0508	0.0487	0.0040
AVG	0.0474	0.0481	0.0462	0.0472	**0.0035**
Test SD	0.0032	0.0035	0.0041	**0.0036**	

The mean coefficient of friction at 100,000 cycles (0.047) was significantly greater than that obtained at 10,000 cycles (0.037) (unpaired t-test, $p<0.05$). This may be attributed to the presence of wear particles generated after the longer period of component articulation [17,19]. However, the coefficient of friction obtained by Mazzucco *et al*. [29] using pin-on-flat polyethylene on metal testing was 0.067 ± 0.012 using distilled water as the lubricant in a non-reciprocating short duration test. While the contact stresses were similar, a smaller indenter with lower loads was used. This indicates that the coefficient of friction measurements are highly sensitive to contact geometry and need to account for the presence of wear particles.

4.7 Conclusions

Periodic variation in friction measurement was obtained in a linear reciprocating test using a spherical metal indenter on a flat UHMWPE counterface with D.I. water as a lubricant. There was a characteristic pattern in the coefficient of friction that corresponded to the reversal in pin motion. An estimate of the coefficient of friction could be reliably obtained taking the average of 30 points about the midpoint between reversals and using the average of three cycles. Test-to-test variation was equivalent to station-to-station variation and this variation was considerably higher than that obtained from cycle-to-cycle. Using this method, the coefficient of friction could be estimated with a standard deviation of 0.005. The method described herein is suitable for the study of the factors affecting friction in total joint replacements.

CHAPTER 5
FRICTION BEHAVIOR OF HYALURONIC ACID SUPPLEMENTED MEDIA

5.1 Introduction

The objective of this chapter is to determine how the friction behaviour of metal on UHMWPE in the context of total knee arthroplasty is affected by lubricating fluid composition. In Chapter 4, a method for the computation of the coefficient of friction using water as the lubricant was determined. This method will be used in this chapter to compute the coefficient of friction of metal on polyethylene with different lubricating fluid composition.

Initial experiments indicated a previously unobserved phenomenon in which the friction dropped with the presence of HA in solution. As a result, validation studies in which HA in D.I. water and HA in albumin solutions were prepared for comparison. In the first, proteins were absent and in the second, other proteins in BCS were absent.

5.2 Friction of Metal on Polyethylene with HA/BCS lubrication

5.2.1 Parameters

The coefficient of friction was determined for CoCr pins on UHMWPE samples. HA incorporated solutions at concentrations of (0-5) mg/mL were used as lubricating environments for friction testing. Friction testing and evaluation was conducted using the methodology described in Chapter 4. Detailed procedures involved for the evaluation of the coefficient of friction are described in the following sections.

5.2.2 Solutions

HA incorporated solutions were prepared using bovine calf serum, BCS as the media. The BCS medium was first prepared by the following additions to 500 mL stock BCS (HyClone™ Laboratories, Logan, UT): 20 mL of 0.2 micron filtered 15.4% EDTA solution (VWR Canlab™, Mississauga, ON) as a chelating agent to calcium, 5 mL of hydrated Fungizone and 3 mL of Penicillin Streptomycin (Invitrogen™ Canada Inc., Burlington, ON) as antimicrobial agents. BCS with protein concentration of 34 mg/mL was obtained (since this concentration was found in fluid at TKA revision arthroplasty [8] [5]) by dilution with 0.2 micron filtered D.I. water. Filtration was conducted using sterile polyethersulfone filters (Nalgene®; Thermo Fisher Scientific, Rochester, NY).

Sodium hyaluronate (M_w = 2.02 MDa), (Genzyme™, Cambridge, MA) was used to make HA/BCS with HA concentrations of 1, 2, 3, 4 and 5 mg/mL. These concentrations

were used to offer a wide range of HA content and are within periprosthetic and normal fluid concentrations.

5.2.3 Pin Polishing

Three tests were conducted for each experimental condition using three different channels. The CoCr pins were polished prior to each test to a mirror finish. Pin polishing was conducted using the procedure outlined in Section 4.3.1.

5.2.4 Sample Preparation

The samples were cut from an UHMWPE sheet (McMaster Carr, Aurora, OH). They were prepared according to the procedure outlined in Section 4.3.2.

5.2.5 Instrumentation, Test Conditions, and Data Acquisition

An AMTI Ortho-Pod^{TM} six station pin on disc friction and wear testing machine was used in this study. Test conditions and data acquisition details used in this study are outlined in Sections 4.2.2 and 4.4 respectively.

5.3 Validation Studies

5.3.1 Rheological Characterization

Solutions

HA incorporated solutions were prepared using D.I. water as the medium. Sodium hyaluronate (M_w = 2.02 MDa), (Genzyme^{TM}, Cambridge, MA) was used to make HA/D.I. water solutions with HA concentrations of 1, 2, 3, 4 and 5 mg/mL.

Experimental

Steady shear rheology evaluations were conducted with a TA Instruments AR 2000^{TM} rheometer (TA Instruments, New Castle, DE). Details of equipment operation and data model fitting are outlined in Section 3.2.2.

5.3.2 Friction of Metal on Polyethylene with HA/D.I. Water Lubrication

Solutions

HA incorporated solutions were prepared using D.I. water as the medium. Sodium hyaluronate (M_w = 2.02 MDa), (Genzyme^{TM}, Cambridge, MA) was used to make HA/D.I. water solutions with HA concentrations of 1, 2, 3, 4 and 5 mg/mL.

Experimental

Pin polishing, sample preparation, instrumentation, data acquisition and analysis were performed according to details outlined in Sections 5.2.3, 5.2.4, and 5.2.5 respectively.

5.3.3 Friction of Metal on Polyethylene with HA/Albumin Lubrication

Solutions

Sodium hyaluronate (M_w = 2.02 MDa), (Genzyme^{TM}, Cambridge, MA) was used to make HA/Albumin solutions with HA concentrations of 1 and 2 mg/mL. Albumin solutions were prepared in phosphate buffered saline and the concentration of albumin was equivalent to that in BCS.

Experimental

Pin polishing, sample preparation, instrumentation, data acquisition and analysis were performed according to details outlined in Sections 5.2.3, 5.2.4, and 5.2.5 respectively.

5.3.4 Stribeck Analysis

The Stribeck analysis is typically used to determine the mode of lubrication at which bearing systems operate [138]. A schematic representation of the Stribeck curve is given in Figure 5.1. The coefficient of friction is plotted against the Sommerfeld number (z) given by the following equation [138]:

$$z = \frac{\eta u}{L} \tag{5.1}$$

where u is the velocity, η is the lubricant viscosity and L is the load applied [138].

In boundary lubrication (BL), the coefficient of friction is constant, while in mixed lubrication (ML), the coefficient of friction decreases with the Sommerfeld number (Figure 5.1). An increase in the coefficient of friction with increased Sommerfeld number is indicative of fluid film lubrication (Figure 5.1) [138].

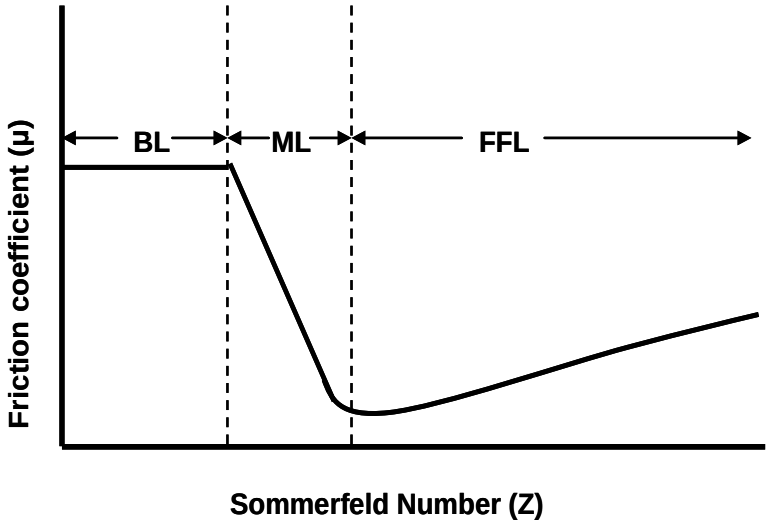

Figure 5.1 Schematic Stribeck curve illustrating the various regimes.

5.4 Experimental results and analysis

5.4.1 Friction of Metal on Polyethylene with HA/BCS lubrication

The coefficient of friction determined for HA/BCS solutions is shown in Figure 5.2. The highest coefficient of friction was obtained with BCS in the absence of HA. This coefficient of friction decreased with HA concentration up to a concentration of 1mg/mL in the BCS medium.

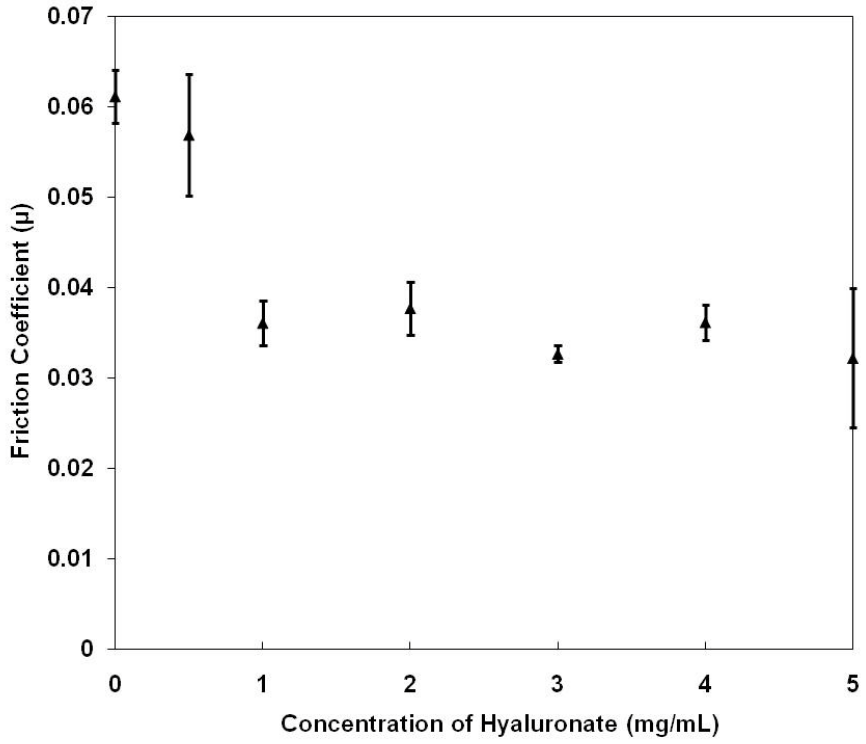

Figure 5.2 Coefficient of Friction vs. HA concentration with HA/BCS Lubrication. Error bars represent the standard deviation.

It is evident that the concentration of HA above 1mg/mL had no effect on the coefficient of friction as the values at these concentrations were nearly identical. In knee replacements, the concentration of HA is highly variable from patient to patient [4], and hence values of HA concentration could fall well below 1 mg/mL. In primary knee replacement, the concentration of HA is 1.3 ± 0.5 mg/mL, while that in revision knee replacement it is 0.9 ± 0.4 mg/mL [8]. In knee replacements with lower HA concentrations, post injection *in vivo* with HA may be a promising procedure that could decrease friction.

As indicated in Chapter 3, the steady shear viscosity and the viscoelastic properties of BCS solutions with different HA concentrations are considerably different. Specifically, the viscosity and elasticity are enhanced with increased HA concentrations. The absence of concentration dependency of the coefficient of friction shown in Figure 5.2 indicates an absence of viscous and elastic fluid properties effect on the friction behaviour of the CoCr pin on the UHMWPE sample articulation.

5.4.2 Validation Studies

Rheological Properties of HA/D.I. Water Solutions

In order to further investigate whether the change in friction is due to a hydrodynamic effect or due to the presence of proteins, solutions of HA were prepared in D.I. water to obtain a lubricating environment where proteins were absent. The steady shear

rheological properties of HA/D.I. water solutions of varying HA concentrations are shown in Figure 5.3.

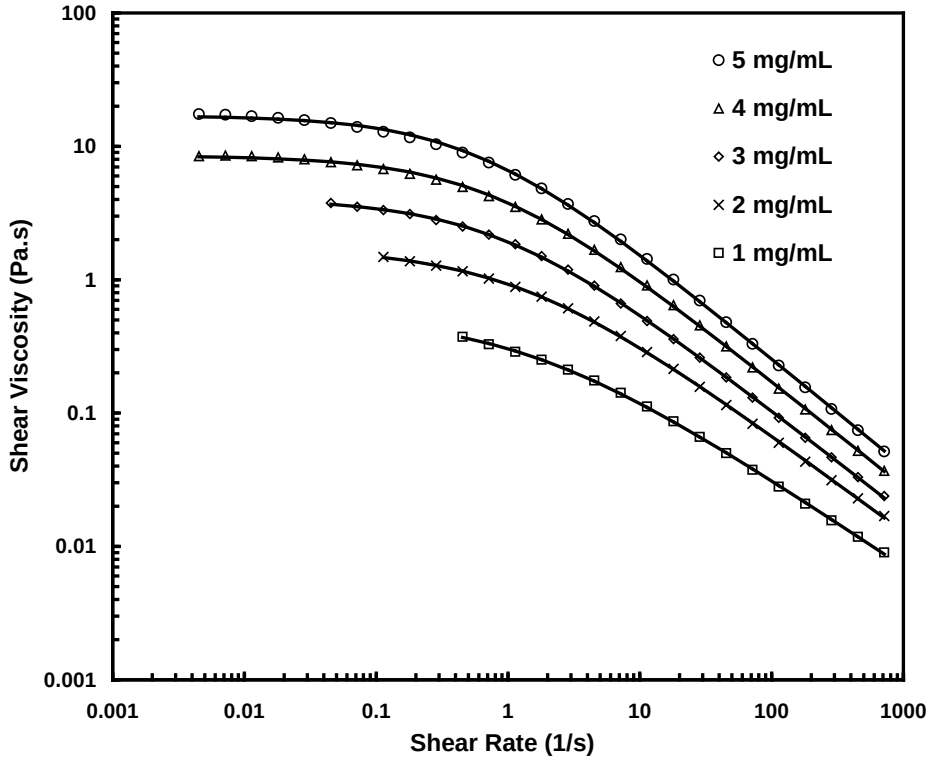

Figure 5.3 Viscosity vs. shear rate data for HA/D.I. water solutions containing different concentrations of HA at 37°C. For each solution, the average of three trial aliquots is plotted. Solid lines indicate the modified Cross model fits.

As observed with HA/BCS solutions, at low shear rates, higher viscosity is observed where molecules are highly entangled and most resistant to flow. At higher shear rates, the viscosity decreases with shear rate due to molecular disentanglement and alignment in the shear field. The modified Cross model fits are also shown in Figure 5.3 and all

parameters are summarized in Table 5.1. Also, as observed with HA/BCS solutions (Chapter 3), with increased HA concentration in the solution, the viscosity and the extent of shear thinning increases. This is also reflected by the corresponding values of the zero shear rate viscosity and the flow index, n, determined by the modified Cross model. Moreover, as the concentration of HA increases in D.I. water, higher relaxation times (λ_r) are observed. Higher relaxation times are indicative of the time needed for chains to disentangle (Table 5.1).

Similar rheological behaviour has been obtained with the increase in HA concentration in saline and in a phosphate buffered solution [60,74]. These results show that a similar mechanism of entanglement network formation exists for HA in D.I. water as that observed for HA in BCS. With increased concentration, a transient entanglement network arises [73,74], which is stabilized by intermolecular associations of hydrogen bonding and non-covalent interactions. The presence of a network gives rise to an enhanced viscosity and a pronounced extent of shear thinning [60,74,76].

Table 5.1 Modified Cross model estimates for each parameter for HA/D.I. water solutions at 37°C.

Solution	η_o **(Pa.s)**	λ_r **(s)**	**n**
(1mg/mL)	0.538	0.681	0.337
(2mg/mL)	1.721	0.810	0.270
(3mg/mL)	4.059	1.150	0.231
(4mg/mL)	8.486	1.334	0.207
(5mg/mL)	16.996	1.746	0.189

Friction of Metal on Polyethylene with HA/D.I Water Lubrication

The coefficient of friction for HA/D.I. water solutions is depicted in Figure 5.4. Irrespective of the concentration of HA in solution, there was no observed difference in the value of the coefficient of friction. Although the rheological properties of these solutions are significantly different, the friction behaviour is nearly identical.

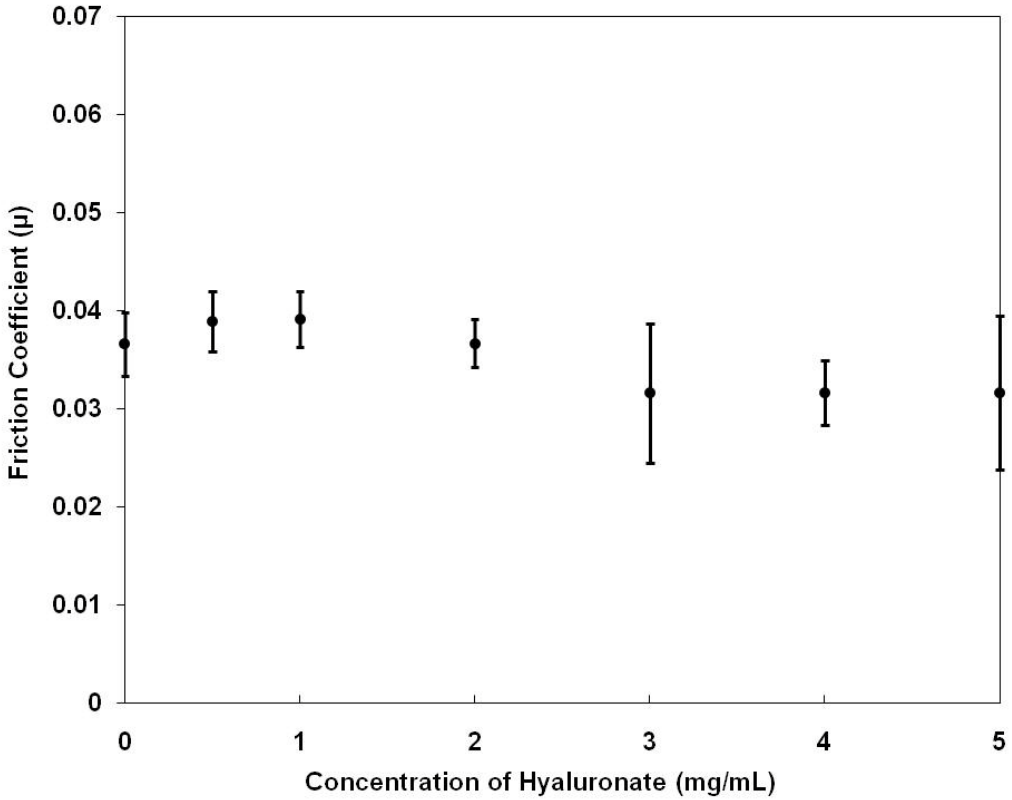

Figure 5.4 Coefficient of Friction vs. HA concentration with HA/D.I. water Lubrication. Error bars represent the standard deviation.

Friction of Metal on Polyethylene with HA/Albumin Lubrication

Bovine serum is composed of many proteins. Albumin is the predominant protein in BCS at 46.9% of the total protein present in the lot used in this study. In order to further isolate the protein effect on the coefficient of friction, model solutions of HA supplemented albumin in phosphate buffered saline were prepared and the coefficient of friction of CoCr pins on UHMWPE samples was determined.

In Figure 5.5 the coefficient of friction was determined for three tests using CoCr pins articulating against UHMWPE samples. Similar friction behaviour was observed with albumin solutions to that obtained with bovine serum solutions. With HA/Albumin solutions at 1mg/mL HA, the coefficient of friction dropped. The coefficient of friction obtained with HA/Albumin solutions at HA concentration of 2 mg/mL was similar to that obtained for HA/Albumin solutions at HA concentration of 1 mg/mL.

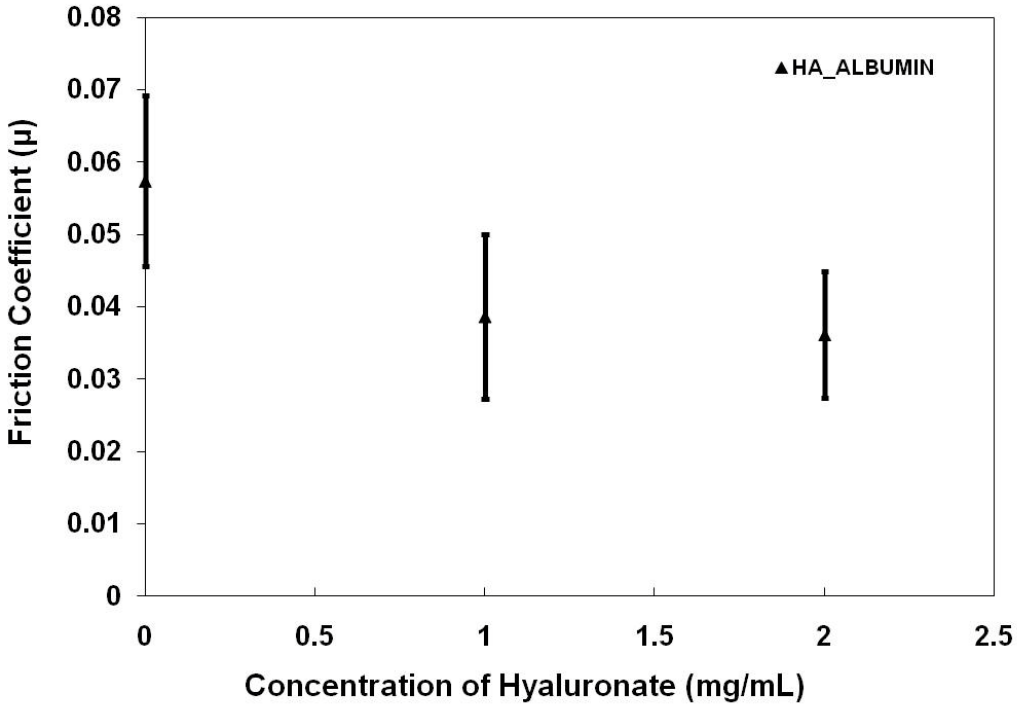

Figure 5.5 Coefficient of Friction vs. HA concentration with HA/Albumin Lubrication. Error bars represent the standard deviation.

The average coefficient of friction obtained for HA in albumin lubrication (Figure 5.5) is similar to that obtained for HA in BCS lubrication (Figure 5.2). The difference in the average coefficient of friction between the three albumin solutions (Figure 5.5) is not statistically significant, (unpaired t-test, $p>0.05$). Albumin alone may not contribute significantly to the increase in the coefficient of friction; however, other proteins in serum may synergistically interact to increase its value.

Stribeck Analysis

The Sommerfeld number for HA/BCS and HA/D.I. water lubrication at HA concentrations of 1-5 mg/mL was determined. The Sommerfeld number (z) given by the following equation [138]:

$$z = \frac{\eta u}{L} \tag{5.1}$$

where u is the velocity, η is the lubricant viscosity and L is the load applied [138].

Since HA solutions are shear thinning, the viscosity is not constant but requires determination by the operating shear rates. Using the modified Cross model predictions previously determined for HA/BCS solutions (Chapter 3) and those obtained for HA/D.I. water (Table 5.1), the viscosity at a shear rate of 100,000 s^{-1} was estimated and the corresponding Sommerfeld number was determined. This shear rate was chosen since shear rates of this magnitude are representative of those experienced in joint replacements [12]. By plotting the coefficient of friction versus the Sommerfeld number, the Stribeck plots for the CoCr alloy on the UHMWPE sample with HA/ BCS lubrication and with

HA/D.I. water lubrication are depicted in Figure 5.6 and Figure 5.7, respectively. No trend was observed between the coefficient of friction and the Sommerfeld number for HA/BCS and for HA/D.I. water lubrication. It is clear from Figure 5.6 and Figure 5.7 that there is a lack of a hydrodynamic effect on the lubrication mechanism at the articulating surfaces and boundary lubrication is the mode of lubrication for the CoCr alloy pin articulating on the UHMWPE sample. Furthermore, the observed coefficients of friction are an order of magnitude larger than those expected for hydrodynamic lubrication.

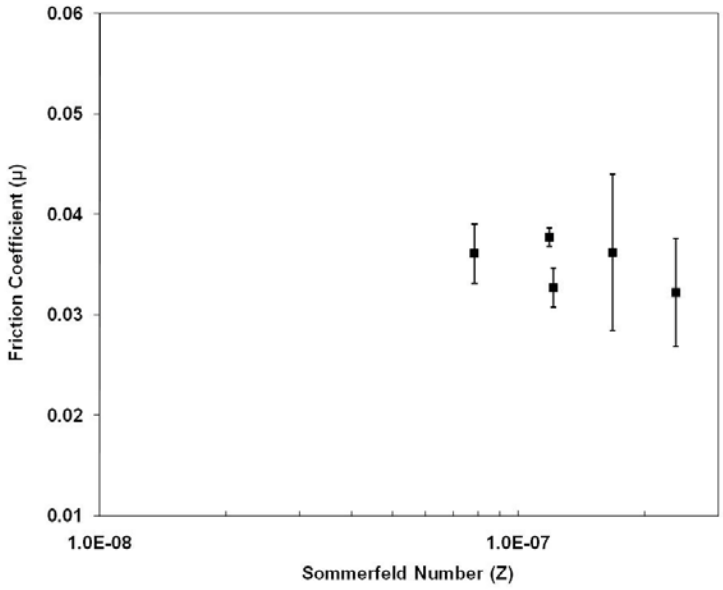

Figure 5.6 Stribeck plot for HA/BCS Lubrication. Error bars represent the standard deviation.

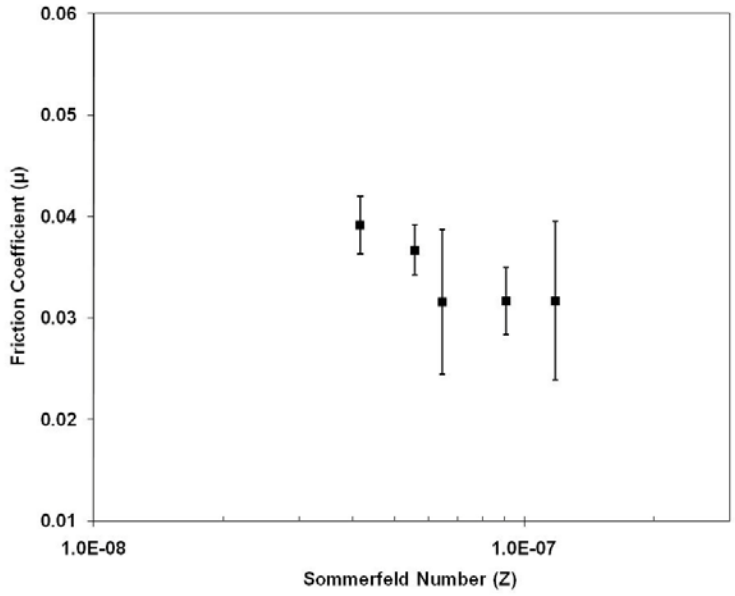

Figure 5.7 Stribeck plot for HA/ D.I. water Lubrication. Error bars represent the standard deviation.

The ideal Stribeck plot for conventional total knee replacements would illustrate a mixed lubrication mode of operation, where the coefficient of friction decreases with the Sommerfeld number [31]. Using carboxymethyl cellulose as the lubricant, Flannery *et al*. [31] observed that not all CoCr on UHMWPE knee replacements tested displayed a Stribeck plot close to the ideal behaviour of mixed lubrication. For those knee replacements that showed behaviour indicative of mixed lubrication, a weak correlation was obtained between the decreasing coefficient of friction with increasing Sommerfeld number. The overall Stribeck plot which depicted the average of the total knee replacement Stribeck plots displayed no observable trend between the coefficient of friction and the Sommerfeld number.

Carboxyemethyl cellulose is a water soluble polysaccharide which yields similar rheological properties to synovial fluid in solution [95]. Although the chemical properties of carboxymethyl cellulose are different from HA, it resembles HA in its ability to form entanglement networks and its rheological properties are similar to those of HA in solution [119]. The Stribeck plot behaviour obtained with carboxymethyl cellulose lubrication is in agreement with that observed in Figure 5.6 and Figure 5.7 with HA incorporated solutions lubrication. No trend was observed between the coefficient of friction and the Sommerfeld number.

5.5 Comparison of Friction of Metal on Polyethylene with HA/BCS, HA/D.I. water, and HA/Albumin Lubrication

It is important to note that while no rheological effects on friction were observed for HA/BCS and HA/ D.I. water solutions, both displayed different behaviour for low HA concentrations. The friction behaviour of the articulating surfaces in both the HA/BCS solutions and the HA/D.I. water solutions is plotted in Figure 5.8.

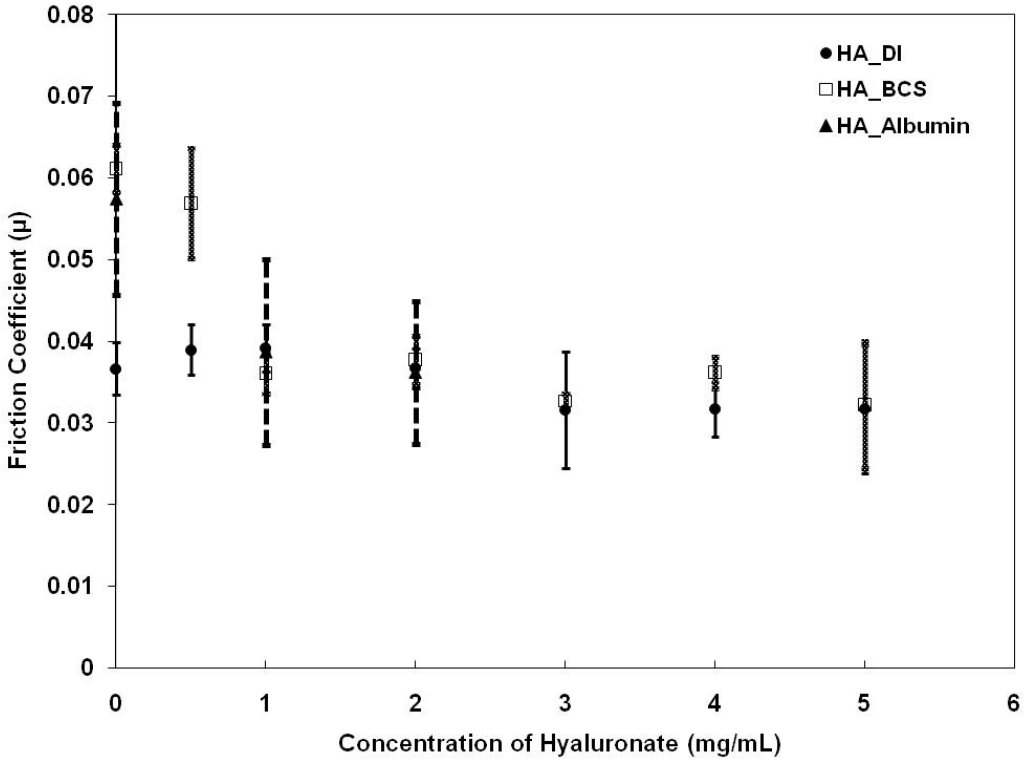

Figure 5.8 Coefficient of friction vs. HA concentration with HA/BCS, HA/D.I. Water and HA/Albumin Lubrication. Error bars represent the standard deviation.

The friction behaviour of the articulating CoCr pin on the UHMWPE sample was higher for BCS (0mg/mL HA) as opposed to D.I. water (0mg/mL HA). Using a pin on disc tribometer, Yao *et al*. [141] have shown that changing from distilled water to diluted BCS as a lubricant led to increased friction for different polymeric bearing materials, even though the viscosities of these two lubricants are nearly equal. Moreover, the coefficient of friction of human synovial fluid was lower than that of bovine synovial fluid despite the fact that both have comparable rheological properties.

A distinct difference in the coefficient of friction behaviour is observed in the BCS and the D.I. water media at the 0 – 1 mg/mL HA concentration range. In D.I. water, there is no significant difference in the friction behaviour, while in BCS, the presence of HA led to a decrease in friction. The major difference in composition between these two media is the presence of proteins in BCS, namely albumin and γ-globulin. Their presence in BCS and the possibility of their synergistic interaction could be contributing to the increase in the coefficient of friction.

Following friction testing, BCS solutions with the low HA concentration were more turbid than those with higher HA concentration, likely due to protein denaturation. Complexation with HA may be responsible for protection from denaturing and their preservation in the soluble state. Further investigation of the characteristics of lubricating fluid before and after friction testing is conducted in Chapter 6.

5.6 Conclusions

The coefficient of friction of HA/BCS solutions decreased as the concentration of HA increased in solution from 0-1mg/mL. Above HA concentration of 1mg/mL, no significant difference in the coefficient of friction was observed. For HA/D.I. water solutions, the coefficient of friction was almost identical irrespective of the HA concentration in solution. Moreover, the significant difference in the rheological properties of HA/D.I. water solutions did not translate into differences in the obtained values of the coefficient of friction. The Stribeck analysis revealed that the coefficient of

friction of HA/BCS and HA/D.I. water solutions was not governed by hydrodynamic conditions. The friction behaviour of metal on polyethylene in HA/Albumin solutions was similar to that obtained with HA/BCS solutions, verifying that the decrease in friction may be due to a protein effect. This is further explored in Chapter 6.

CHAPTER 6
CHARACTERISTICS OF LUBRICATING MEDIA BEFORE AND AFTER FRICTION TESTING

6.1 Introduction

In Chapter 5, no effect of rheology on the coefficient of friction was observed for solutions with different HA concentration. In this chapter, the composition and characteristics of the lubricating medium, specifically the presence of proteins was investigated in order to better understand the mechanism of friction behaviour. Hyaluronate is bound to protein [65,66], however, the protein component does not influence the rheology of the HA-protein complex. Complexation may affect the tribological properties of orthopedic components.

Several studies have investigated the effect of the presence of proteins in the lubricating medium on friction in joint arthroplasty. In studies by Flannery *et al.* [31], the coefficient of friction obtained from a 10 mg/mL HA viscosupplement was generally lower than that obtained from the 5 mg/mL HA in BCS medium albeit the difference was not statistically significant. Although, the concentration of HA is different in the two lubricating environments, and hence these could not be properly compared, the authors postulated that this variation could be due to the contact and rubbing of proteins at the articulating surfaces introduced through the BCS medium [31]. No clear indication was made as to

the state of the proteins whether they are present in the soluble or the insoluble denatured state.

Brockett *et al.* [142] studied the effect of serum concentration on the friction behaviour of different articulating material combinations including CoCrMo on polyethylene in a hip simulator using water, 25% serum and 100% serum. The coefficient of friction increased with protein concentration, with water (0% serum) offering the lowest coefficient of friction.

Studies by Scholes and Unsworth [128] suggested that proteins on the surfaces of metal on polyethylene joint prostheses were contributing to the friction observed via protein to protein rubbing at the contact zones. CoCrMo on polyurethane and alumina on alumina joints operated under fluid film lubrication when CMC fluids were used. The coefficient of friction increased significantly when bovine serum was used as the lubricant. The authors postulated that with bovine serum as the lubricant, the proteins adsorbed on the surfaces breakdown the fluid film formed by the microelastohydrodynamic lubrication, causing protein to protein rubbing. In metal on polyethylene systems lubricated with BCS, the protein adsorbed on to the metal and the UHMWPE surface resulted in some protein to protein rubbing and also some CoCrMo on UHMWPE contact.

Scholes and Unsworth postulated that the adsorbed protein layer is beneficial in terms of wear but can either increase or decrease the friction between the contacting surfaces. For metal on metal, the introduction of proteins by bovine serum decreased the friction between the bearing surfaces. It was postulated that the protein layer protects the surfaces to a certain degree, reducing metal to metal contact (which creates high friction). This contact is replaced by a mixture of metal to metal contact and protein to protein contact which will result in lower friction. However, no clear distinction was made regarding the nature of the proteins involved whether present in the soluble or insoluble denatured state. However, in studies by Gispert *et al.* [130], the adsorbed protein layer was thought to decrease the friction in metal on polyethylene pin-on-disk friction measurements using bovine serum albumin in salt solution as a lubricant. Gispert *et al.* [130] attributed the lack of reduction in the coefficient of friction in alumina systems by the instability of the adsorbed protein layer on the more hydrophilic alumina surface. The surface properties of UHMWPE, alumina, CoCrMo and stainless steel were determined by measurement of contact angles and calculating the work of adhesion. The most hydrophobic surface was UHMWPE, followed by stainless steel, followed by CoCr, followed by alumina.

The objective of this chapter was to investigate the change in the nature of the lubricating solutions containing protein before and after friction testing and to determine the nature of precipitates formed during the test.

6.2 Methods and Materials

Samples were collected following the friction test and were stored overnight. Following storage, precipitates were formed in BCS solutions but were absent in the BCS solutions with HA concentration above 3mg/mL.

Absorbance measurements were conducted using a Cary 100 Varian™ Spectrophotometer for BCS solutions at HA concentrations of 0, 1 and 3 mg/mL. These measurements were also conducted for albumin solutions at HA concentrations of 0, 1, and 2 mg/mL. Absorbance was recorded for a range of wavelengths (300-700 nm). The absorbance at 536 nm was used to conduct solution comparisons; this wavelength has been previously used to characterize protein denaturation [143].

Particle size measurements by dynamic light scattering were conducted for an albumin solution before and after friction testing and for a BCS solution before friction testing. Particle size measurements were conducted using the Malvern Instruments Zetasizer™ Nano series. It measures particles in the size of 0.6 nm to 6 microns using dynamic light scattering. Dynamic light scattering measures Brownian motion and relates this to particle size. Particles are illuminated with a laser and the intensity of fluctuations in the scattered light is analyzed.

The denatured proteins that were suspended within solution following the test precipitated upon storage. Samples were collected for HA/BCS solutions and for

HA/Albumin solutions following friction tests and were centrifuged. The supernatant was removed and the precipitate was washed with D.I. water, centrifuged and the supernatant was subsequently removed. Washing, centrifugation, and supernatant removal were conducted three times.

A ninhydrin based test was conducted to determine if the precipitates remaining after supernatant removal contain protein. In the presence of free primary amines, an intensive blue colour is generated with the ninhydrin test. The reaction scheme of the ninhydrin test is illustrated in Appendix C, Section C.1.

6.3 Experimental results and analysis

It was observed that following friction testing, BCS solutions with low HA concentrations were more turbid than those with higher HA concentrations. These precipitates are likely proteins that were denatured during the test. It is likely that these solid denatured proteins are contributing to the coefficient of friction by rubbing at the interface between the metal and polyethylene. If the turbidity of the solution decreases with increased HA concentration, then the relative concentration of proteins in their denatured state to those in their soluble state decreases with HA concentration. This could be due to the ability of HA to complex with proteins [144]. Such complexation allows proteins to be anchored to the large chains of HA and may protect them from unfolding. Figure 6.1 illustrates photos of samples obtained before and after the test which also indicate an increase in turbidity following friction testing. Moreover, the 0 mg/mL

HA/BCS solution is the most turbid solution. In order to better quantify this effect absorbance and dynamic light scattering were conducted.

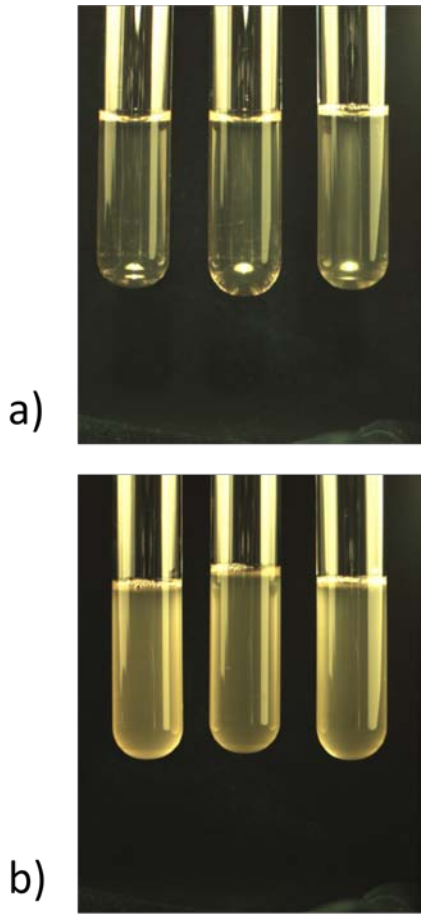

Figure 6.1 Photos of BCS solutions before the test (0mg/mL HA, 1 mg/mL HA, and 3mg/mL HA respectively) b. Photos of BCS solutions after the test (0mg/mL HA, 1 mg/mL HA, and 3mg/mL HA respectively).

6.3.1 Absorbance measurements

The absorbance at 536 nm of HA/BCS solutions was determined before and after the friction testing. After friction testing, absorbance increased for all solutions. The difference in absorbance obtained after the test from that obtained before the test is recorded in Table 6.1.

Table 6.1 Difference between absorbance obtained after friction testing from that obtained before friction testing for BCS and albumin solutions.

Solution	Test 1	Test 2	Test 3	AVG	SD
0 mg/mL HA/BCS	1.64	1.63	1.54	1.61	0.06
1 mg/mL HA/BCS	1.28	1.24	1.18	1.23	0.05
3 mg/mL HA/BCS	1.12	1.25	1.09	1.15	0.08
Albumin	0.63	0.60	0.57	0.60	0.03
1mg/mL HA/Albumin	0.27	0.28	0.30	0.28	0.01
2 mg/mL HA/Albumin	0.35	0.25	0.31	0.30	0.05

For BCS solutions, the increase in absorbance and turbidity was more pronounced for solutions at 0 mg/mL HA for the three tests as opposed to solutions with higher HA concentration. The mean difference in absorbance for 0 mg/mL HA/BCS (1.61) was significantly greater than that obtained from the 1 mg/mL HA/BCS and the 3 mg/mL HA/BCS solutions (1.23 and 1.15 respectively) (unpaired t-test, $p<0.05$). However, the difference between the average difference in absorbance obtained from the 1 mg/mL HA/BCS and the 3 mg/mL HA/BCS solutions was not statistically significant (unpaired t-test, $p>0.05$).

The absorbance at 536 nm of albumin solutions was also determined before and after the friction testing. As observed with the BCS medium, the absorbance increased for all solutions after the test and the difference was higher for albumin solutions compared to the HA supplemented albumin solutions (Table 6.1). The mean difference in absorbance for albumin solution (0.60) was significantly greater than that obtained from the 1mg/mL HA/Albumin and the 2mg/mL HA/Albumin solutions (0.28 and 0.30 respectively) (unpaired t-test, $p<0.05$). However, the difference between the average difference in absorbance obtained from the 1mg/mL HA/Albumin and the 2mg/mL HA/Albumin solutions was not statistically significant (unpaired t-test, $p>0.05$). Absorbance measurements were also determined for these solutions relative to an HA solution of 1mg/mL and the same deviation was obtained as that depicted in Table 6.1.

6.3.2 Dynamic light scattering

Dynamic light scattering measurements of size were conducted for BCS before the test. Figure 6.2 illustrates the different size peaks obtained for BCS. The peaks obtained correspond to the different proteins present in BCS. It is important to note that measurements were conducted for BCS solutions after the test and for HA/BCS solutions before and after the test. However, no reliable data were obtained due to the polydisperse nature of these solutions leading to multiple peaks and back scattering.

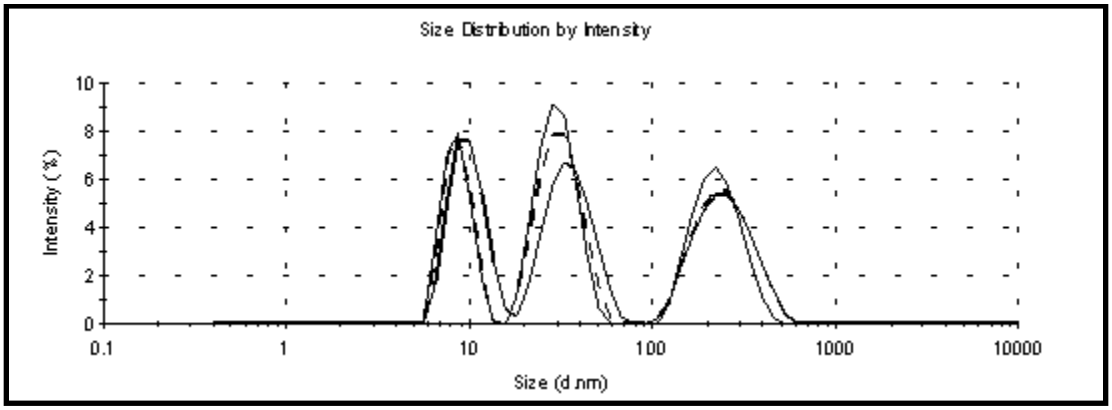

Figure 6.2 Dynamic light scattering size measurements of a BCS solution before friction testing.

Figure 6.3 reveals the dynamic light scattering size measurements for albumin in buffer solution. One peak is observed at 8 nm, which corresponds to the first peak obtained in Figure 6.2.

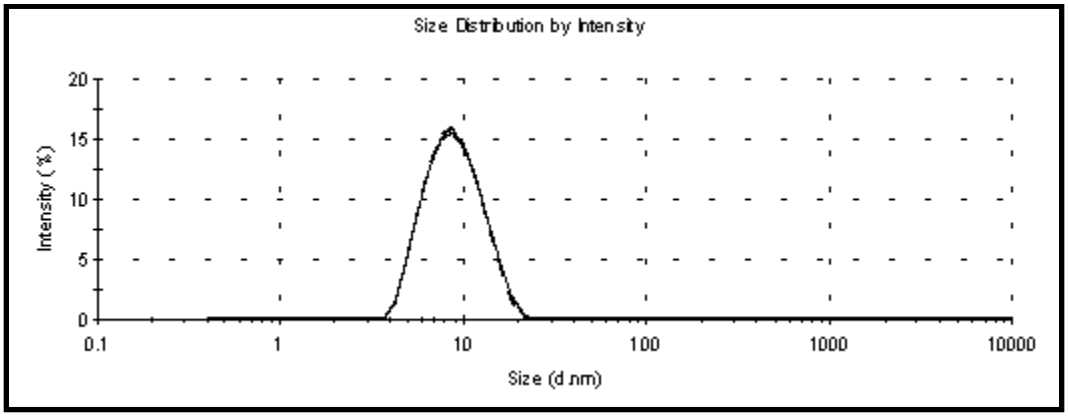

Figure 6.3 Dynamic light scattering size measurements of an albumin solution before friction testing.

Figure 6.4 illustrates the dynamic light scattering size peaks for albumin after friction testing. The first peak is at 8 nm which corresponds to undenatured soluble albumin. The peak obtained at 1000 nm is indicative of large aggregates of albumin, which are possibly due to denaturation and aggregation following the friction test.

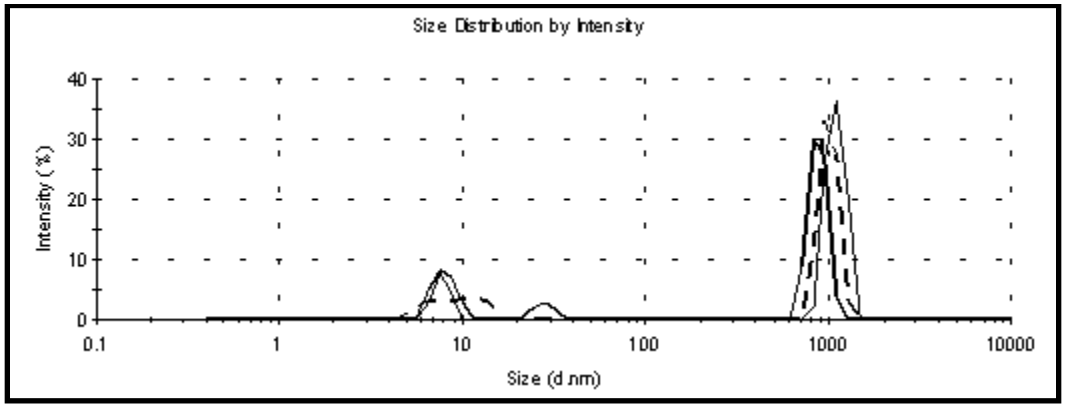

Figure 6.4 Dynamic light scattering size measurements of an albumin solution after friction testing.

6.3.3 Precipitate Determination

The precipitates from samples of BCS and from samples of 1mg/mL HA/BCS that were collected following friction testing gave a positive intensive blue colour while control samples of ninhydrin solution and of HA/D.I. water solution did not (Appendix C, Section C.2.1). The ninhydrin test was also conducted on albumin solutions where the precipitates from samples of albumin and samples of 1mg/mL HA/BCS that were collected following friction testing also gave a positive intensive blue colour (Appendix

C, Section C.2.2). Again, control samples of ninhydrin solution and of HA/D.I. water solution did not.

6.4 Discussion

6.4.1 Protein Denaturation

The characterizations of absorbance and particle size reveal that the nature of proteins before and after the test is altered and this could have an effect on the coefficient of friction observed for solutions of BCS and albumin.

Heuberger *et al.* [145] found that unfolded proteins adsorb unto hydrophobic UHMWPE and increase friction. Increase in friction correlated well with the relative decrease in native (folded) protein. Adsorbed denatured proteins are less efficiently hydrated, and the authors postulated that this could decrease their ability to act as low shear modulus boundary lubricants. By conducting an optical adsorption study, the authors found that hydrophobic surfaces preferentially adsorb denatured proteins from a mixed solution. The authors indicated that the observed differences in boundary lubrication are linked to the different capacities of the adsorbed film to bind water. They suggested that using hydration water bound to adsorbed hydrophilic molecules may be promising in achieving bio-lubricated systems beyond arthroplasty.

Sodium hyaluronate is a highly hydrophilic macromolecule with polyanionic character [146]. Moreover, it has high capacity for water retention [147]. As mentioned before, in

studies by Heuberger *et al*. [145], unfolded denatured proteins adsorbed onto the hydrophobic surfaces via strong hydrophobic interactions. It is likely that in the BCS solutions, the denatured hydrophobic proteins adhere or adsorb on the hydrophobic polyethylene surface. This adsorbed solid protein layer at the interface is likely to be less hydrated, and hence less able to act as a low shear modulus lubricant leading to the rise in the coefficient of friction. The presence of HA, being hydrophilic, may interfere with the adsorption of the hydrophobic proteins on the hydrophobic articulating surfaces. This poses the question of what the effect of HA is in the process of protein denaturation and this will be explored in Section 6.4.2.

Boundary lubrication could be governing the difference in the friction behaviour between HA/BCS and HA/D.I. water solutions at HA concentration of 0-1 mg/mL (Figure 5.8). Compared to the adsorbed solid denatured proteins on the articulating surface, the water retaining HA incorporated solutions could be acting as more effective boundary lubricants decreasing the coefficient of friction by their ability to form low shear-strength lubricating layers on the articulating surfaces.

HA complexation with albumin in the lubricating channel could prevent albumin molecules from being readily available at the interface of the articulating surfaces. HA through its interaction with albumin molecules anchor these protein molecules preventing them from reaching the interface and hence from mechanical and thermal denaturation.

The presence of solid precipitated proteins at the interface could interfere with boundary lubrication and hence lead to an increase in friction. It is likely that the amount of precipitated protein at the interface is reduced (when HA is complexed with albumin) and is replaced by hydrated HA layers, which leads to the reduction in the coefficient of friction.

6.4.2 HA-Protein Complexation

HA is usually combined with proteins in living tissue [148]. At physiological pH (7.4) HA is negatively charged. The negative charge of HA is attributed to the anionic carboxylate group ($–COO^-$) [148].

The binding of polyelectrolytes to proteins is predominantly governed by electrostatic forces [149]. An acid polysaccharide and a protein interact electrostatically to form soluble and/or insoluble complexes [150]. In neutral or weak acidic pH, polyanions and proteins form soluble complexes, and in the more acidic pH region, phase separation occurs [151]. The pI of the protein is the pH where the protein carries no net electric charge. At pH<pI, the net charge of albumin is positive and electrostatic attraction occurs between the positive charge of albumin and the negatively charged HA (pI of albumin: 4.7). However, several studies have shown that binding can often occurs pH>pI for proteins binding to polyanions. This is due to the presence of “charge patches” (Figure 6.5) on the protein surface, opposite in sign to the net protein charge (Z_p) [152]. These charge patches can provide sites for the binding of polyelectrolytes that have the same

sign as Z_p [152]. Polyanions have been found to complex with bovine serum albumin. [152]. The protein's non uniform charge distribution, allows for some regions rich in basic residues to interact strongly with polyanions irrespective of the presence of the net negative charge.

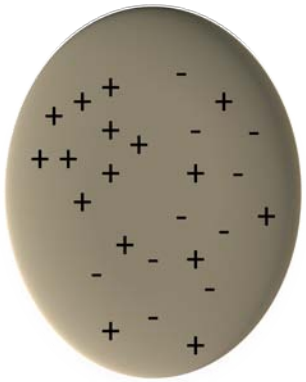

Figure 6.5 Schematic depiction of protein with positive charge patches after Park *et al*. [152].

Although the pH of HA supplemented BCS solutions and albumin solutions was neutral (7.4-7.8), interaction between albumin and HA can still occur due to the presence of positive charge patches.

Complex formation between sodium hyaluronate and BSA involves:

$n(HA\text{-}COO\text{-}Na) + BSA \rightarrow BSA(HA\text{-}COO)_n + nNa^+$ [151]

where n is the number of repeating units, which is also the number of carboxylic acid groups available for binding with albumin [151].

The molecular weight of NaHA is $2.34X10^6$ g/mol, the molecular weight of the repeating unit of HA is 401 g/mol. Therefore, there are 5835 units in one molecule of HA. The concentrations of hyaluronate and albumin are shown in Table 6.2.

Table 6.2 Concentrations of albumin and sodium hyaluronate used.

Albumin (mol/L)	HA (mg/mL)	HA (mol/L)
$1.28X10^{-4}$	1	$4.27X10^{-7}$
$2.49X10^{-4}$	2	$8.55X10^{-7}$

The number of molecules of HA in 1L of solution is calculated by:

Number of Molecules of HA/L

$$= 6.02X10^{23} \times 4.27X10^{-7}\, mol/L = 2.57X10^{17}\, molecules/L$$

The number of molecules of albumin in 1L of solution is calculated by:

Number of Molecules of Albumin/L

$$= 6.02X10^{23} \times 1.28X10^{-4}\, mol/L = 7.71X10^{19}\, molecules/L$$

The number of units of HA in 1L of solution is calculated by:

Number of Units of HA/L

$$= 5835 units \times number\_of\_moleculesofHA/L$$

The number of units of HA/molecules of albumin in solution are calculated by:

Number of Units of HA/Molecule of Albumin

$$= \frac{Number\_of\_unitsofHA/L}{Number\_of\_moleculesofAlbu\,\min/L}$$

The number of units of HA/molecules of albumin in solution were determined for HA supplemented BCS and albumin solutions and are given in (Table 6.3).

Table 6.3 Coefficient of friction and Number of units of HA/molecules of Albumin in Solution.

Solution	μ	Units of HA/Molecules of Albumin
Albumin	0.057	0
Albumin + HA (1mg/mL)	0.039	**19**
Albumin + HA (2mg/mL)	0.036	39
BCS1	0.061	0
BCS1 + HA (1mg/mL)	0.036	**19**
BCS1 + HA (2mg/mL)	0.038	39
BCS2	0.068	0
BCS2 + HA (1mg/mL)	0.063	10
BCS2 + HA (2mg/mL)	0.032	**20**

Irrespective of the HA supplemented medium, considerable reduction in the coefficient of friction occurred when the number of units of HA/molecules of Albumin is between 10 and 20. This is consistent with studies by Xu *et al*. [151] where the number of units of NaHA required to bind with one molecule of bovine serum albumin was found to be 15.

This data suggest that interaction between HA and albumin through intramolecular complexation could be governing the reduction of the coefficient of friction of HA supplemented solutions. This proposed mechanism of interaction is depicted in Figure 6.6.

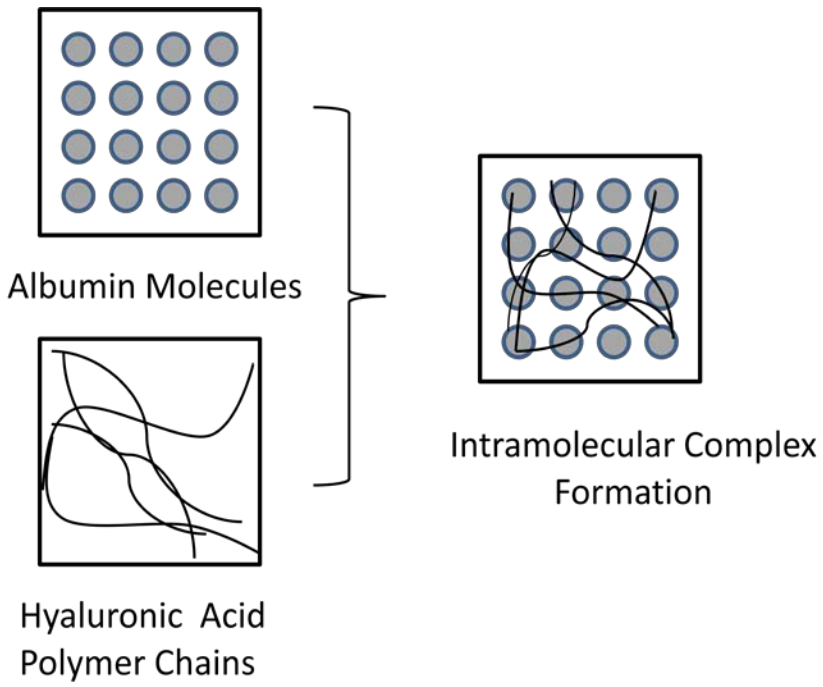

Figure 6.6 Schematic of intramolecular complex formation between hyaluronic acid and albumin molecules after Park *et al*. [153].

6.4.3 Discussion

Wear of knee replacement components has been studied rigorously in order to optimize the performance of these bearings [10,13-28]. Little has been done to evaluate the friction

in these systems [29-31]. Lower friction does not necessarily lead to less wear; the first Sir John Charnley low friction arthroplasty of the hip based on stainless steel femoral component and polytetrafluoroethylene acetabular cup yielded low friction but high wear [154]. Moreover, while a higher level of protein precipitates could lead to the increase in the coefficient of friction, studies have shown that their presence could decrease wear. In simulator testing of hip replacements, protein precipitation in diluted serum was lower than that in more concentrated serum [155]. Also, polyethylene cup wear in diluted serum was greater. The authors suggested that pre-diluted serum minimizes the tendency for a solid layer of precipitated protein to build up between the ball and cup which may artificially protect the polyethylene cup from wear [155].

However, friction measurements could elucidate the mode of lubrication occurring within joint prostheses which can in turn affect the operation of the bearings. Boundary lubrication is the lubrication mechanism occurring between the CoCr pin and UHMWPE sample since the coefficient of friction was not affected by fluid rheology. However, the drop in the coefficient of friction in the presence of HA is likely due to its interaction with proteins. Absorbance and light scattering measurements suggest that complex formation is likely to occur between HA and protein. Albumin alone may not be responsible for the increase in friction, but it is likely that its synergistic interaction with other proteins could lead to the increase in the coefficient of friction.

6.5 Conclusions

Absorbance measurements of HA/BCS solutions suggested that proteins were denatured following friction testing. The extent of denaturation was higher in the absence of HA in solution. Absorbance measurements of HA/Albumin solutions reveal a similar effect. Dynamic light scattering measurements of albumin solutions following the test reveals the presence of larger size particles that could be due to protein denaturation and aggregation. Further testing confirmed that the precipitates formed in the lubricating media after friction testing consisted of protein. A mechanism is proposed where the number of units of HA/molecules of albumin that is required for considerable friction reduction is between 10 and 20.

CHAPTER 7
CONCLUSIONS, SIGNIFICANT CONTRIBUTIONS AND RECOMMENDATIONS FOR FUTURE WORK

7.1 Conclusions

This thesis examines the effect of periprosthetic fluid composition on its rheology and on friction in knee replacement bearings. In Chapter 3, the effect of fluid composition on the steady shear viscosity of HA/BCS solutions was determined. As the concentration and molecular weight of HA increased, the viscosity and the extent of shear thinning increased. Moreover, the elastic properties of solutions were enhanced with the increase in HA concentration and molecular weight. This was revealed through the change in moduli and the decrease in the cross-over frequency. The rheological behaviour of HA/BCS solutions of different concentration and molecular weight was consistent with that of synovial fluid under different pathological conditions.

In order to determine the effect of composition and rheology on the friction properties of total knee replacements, Chapter 4, outlined the method of friction measurement that was developed for a linear reciprocating wear screening machine. Periodic variation in friction measurement was obtained with a spherical metal indenter on a flat UHMWPE counterface using D.I. water as a lubricant. The pattern obtained in the coefficient of friction corresponded to the reversal in pin motion. The coefficient of friction could be

reliably estimated by taking the average of 30 points about the midpoint between reversals and using the average of three cycles. Station-to-station variation was equivalent to test-to-test variation and this variation was considerably higher than that obtained from cycle-to-cycle. The coefficient of friction could be estimated with a standard difference of 0.005 using this method.

The method for the friction estimation was used in Chapter 5 to determine the friction behavior of HA/BCS solutions with different HA concentrations. As the concentration of HA in solution increased in solution from 0-1mg/mL, the coefficient of friction of HA/BCS solutions decreased. No significant difference in the coefficient of friction was observed above HA concentration of 1mg/mL. However, for HA/D.I. water solutions, the coefficient of friction was almost identical irrespective of the HA concentration. Moreover, irrespective of the significant difference in the rheological properties of HA/D.I. water solutions no significant difference in the coefficient of friction was observed. In addition, the Stribeck analysis revealed that the coefficient of friction of HA/BCS and HA/D.I. water solutions was not governed by hydrodynamic conditions. Using a model system of HA in albumin solution, the friction behavior was found to be similar to that observed with HA in BCS solution.

In Chapter 6, the lubricating fluid was characterized before and after friction testing. Absorbance measurements of HA/BCS solutions revealed that the turbidity of solutions

increased following test, which is indicative of protein denaturation. When HA was absent, the extent of denaturation was greater. Similar effects were observed when albumin was used as the medium. Dynamic light scattering measurements of albumin solutions before and after the test indicated the likelihood of protein denaturation; large size particles were observed for samples collected after friction testing. The precipitates formed in samples that were collected following friction testing were likely denatured protein precipitates and this was confirmed by the ninhydrin test.

The presence of denatured solid proteins at the interface between metal and polyethylene could interfere with the formation of boundary lubricating layers, and hence increase the coefficient of friction. The presence of HA in solution affected the level of protein denaturation and precipitation during the test. Complexation between HA and proteins may limit the level of protein denaturation. This could be due to the anchoring of proteins by HA chains such that protein molecules such as albumin were less available at the interface to be mechanically and thermally degraded during the test. A model for the complexation of HA with albumin has been proposed where approximately 10-20 units of HA/molecule of albumin was required to bring about a decrease in the coefficient of friction.

7.2 Significant Contributions

Over the course of this work, several contributions to the advancement of knowledge in the area of knee replacement tribology were made.

First, characterization of the steady shear and the oscillatory shear behavior of HA/BCS solutions provided the understanding of how rheological properties are affected by fluid composition. This step is necessary since further investigation and correlation with tribological properties of joint replacements can be made. This contribution is significant because even though it is well known that the incorporation of HA in solution leads to enhancement of the rheological properties in solution, it is important to investigate how the rheological properties of HA are affected when present in the BCS medium that corresponds to the medium encountered *in vivo* and in *in vitro* wear screening and simulator testing.

Secondly, a method was developed to estimate the coefficient of friction in a reciprocating wear screening machine. Typically this machine is used for wear screening and friction measurements in general are conducted in non-reciprocating motion. The developed method allows the determination of friction in reciprocating motion representative of joint replacements.

Thirdly, the rheology of the fluids investigated did not affect the friction of metal on polyethylene knee replacements. To the knowledge of the author, a rigorous study of the affect of HA concentration and its rheological properties on friction has not been studied. The rheology of the solutions was shown not to affect the coefficient of friction. While HA is currently administered to natural joints, the lack of a rheological effect on friction in prosthetic components studied in this work, may have clinical importance.

Fourthly, the presence of HA affected the level of denaturation and precipitation of proteins after friction testing. HA interacts with proteins and this has been reported in the literature. However, it has not been shown before that this interaction could affect the level of protein precipitation and denaturation. Also, it has not been shown before that the interaction of HA with protein could affect the friction behavior in joint replacements.

7.3 Recommendations for Future Work

I. Examine further the complexation of proteins with HA by using a model system such as albumin. Different concentrations of albumin and HA should be investigated and the effect of each concentration on complex formation should be examined in order to obtain a comprehensive model of HA- protein interaction. It is also important to consider low HA concentrations between 0-1 mg/mL HA and determine the coefficient of friction obtained under lubrication with these solutions.

II. Investigate the effect of molecular weight on the rheology and friction in knee replacement bearings. The characteristics of the HA polymer before and after the friction test could be investigated by determining the molecular weight distribution before and after the test. This could indicate whether polymer degradation occurs during the test.

III. Investigate the adsorption of proteins and HA on the metal and on the polyethylene components. By observing the relative adsorption of each fluid component on each surface, better understanding of protein denaturation could be obtained. Adsorption of proteins on the surface could lead to protein unfolding and denaturation, and it may be possible that the presence of HA interferes with protein adsorption. This could occur in addition to the mechanical degradation of proteins by the applied load and their denaturation and precipitation by frictional heating during friction testing.

IV. Investigate the wear properties of metal on polyethylene using model solutions of albumin and HA. It would be beneficial to determine the effect of each component, albumin and HA separately and in combination on the wear properties. BCS is typically used in wear testing, however, the presence of several components such as other proteins and ions in solution could affect the complexation of proteins with HA.

The results of these wear tests could allow for correlations to be obtained with friction studies that has previously investigated the interaction between HA and protein. Understanding the effect of HA-protein interaction on the wear properties of knee replacement components promotes the study of new clinical approaches to these prostheses.

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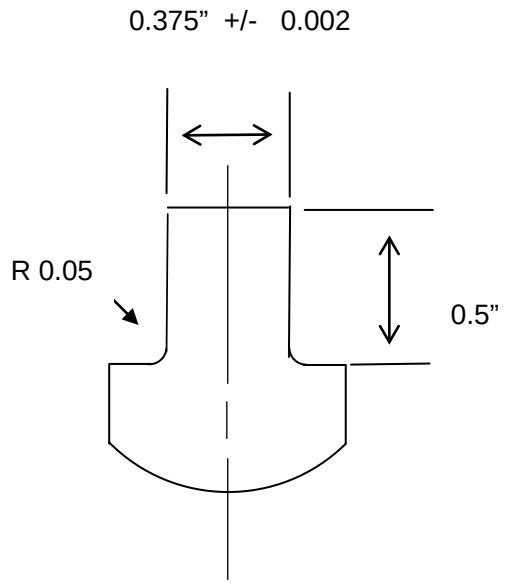
Appendix A

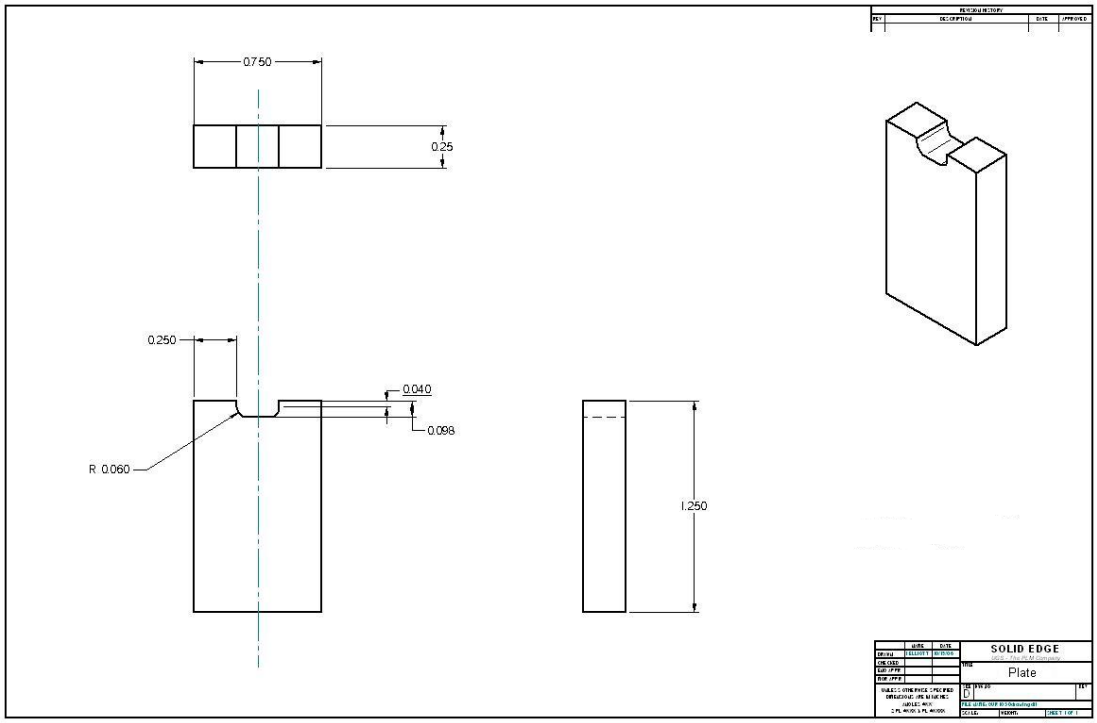
Specimen Geometry

A.1 Pin



A.2 UHMWPE Specimen

Drawing by: Iris Elliot



Appendix B

Procedures

B.1 Bovine Serum Procedure

1. Thaw Bovine Serum and about 5mL of Penicillin Streptomycin at room temperature or in fridge over night.

2. Make EDTA Solution
(Procedure from John Medley)

- add 77.154 g of EDTA to a beaker containing about 0.3 L of D.I. water
- place the beaker on a stirring plate (that has a little magnetic stirring pellet in the beaker)
- monitor the pH level in real time (it will drop as EDTA dissolves)
- add NaOH slowly to increase the pH thus keeping it in the range of 7.5 - 8.0 which is optimum for the dissolving EDTA
- stop adding NaOH when a stable reading of pH = 8.0 is achieved
- resist the temptation to add too much NaOH at one time because the final stable pH might exceed 8.0
- finally add more DI. water to obtain a total volume of 0.5 L

3. Filter Bovine Serum

- Use a vacuum filter apparatus with a 450 nm filter to remove fibrinogen.

4. Rehydrate the fungizone

- Filter D.I. water using a 0.2 micron syringe filter and add 20 mL of this water to the dehydrated fungizone bottle.

5. Filter the EDTA using a 0.2 micron filter

6. Add Fungizone, Penicillin Streptomycin, and EDTA to Bovine Serum

To the 500mL bottle of Bovine serum add

20	mL	EDTA solution
5	mL	hydrated Fungizone
3	mL	Streptomycin

TOTAL 528	mL	of 94.7% serum

B.2 Sample Cleaning Procedure (ASTM F732)

1- Rinse with tap water to remove bulk contaminants.
2- Wash in an ultrasonic cleaner in a solution of 1% detergent for 15 minutes
3- Rinse in a stream of distilled water.
4- Rinse in an ultrasonic cleaner in distilled water for 5 minutes.
5- Rinse in a stream of distilled water.
6- Dry with lint-free tissue.
7- Immerse in methyl alcohol for 3 minutes. This is done to remove the water from the surface layer of the specimen that otherwise tends to evaporate during the weighing process.
8- Dry with lint-free tissue
9- Air-dry in a dust-free environment at room temperature for 30 minutes
10- Store samples in separate sterile containers.

B.3 Metal Polishing Procedure

Step 1 (6 micron Diamond Spray)

1- Use the 6 um diamond air spray bottle for this step of polishing. Shake bottle well.
2- Pour substantial amount of this spray on the cloth circle, around 8 to 10 squirts.
3- Add a little amount of diamond lubricant.
4- Turn motor to low setting
5- Add lubricant as needed.
6- Polish for 3-5 minutes
7- Wash cloth with water and brush extra material away.
8- Wash sample with soap water, rinse with water, and dry with air.
9- Examine surface at 4X magnification in the microscope.

Step 2 (0.05 micron Alumina Solution)

1- Clean cloth surface with water and brush
2- Add alumina solution to cloth surface.
3- While rotating sample and polishing add minute amounts of alumina solution
4- Continue polishing for 3-5 minutes.
5- Wash cloth with water and brush extra material away.
6- Wash with soap water, rinse with water, and dry with air.
7- Examine surface at 4X magnification in the microscope.

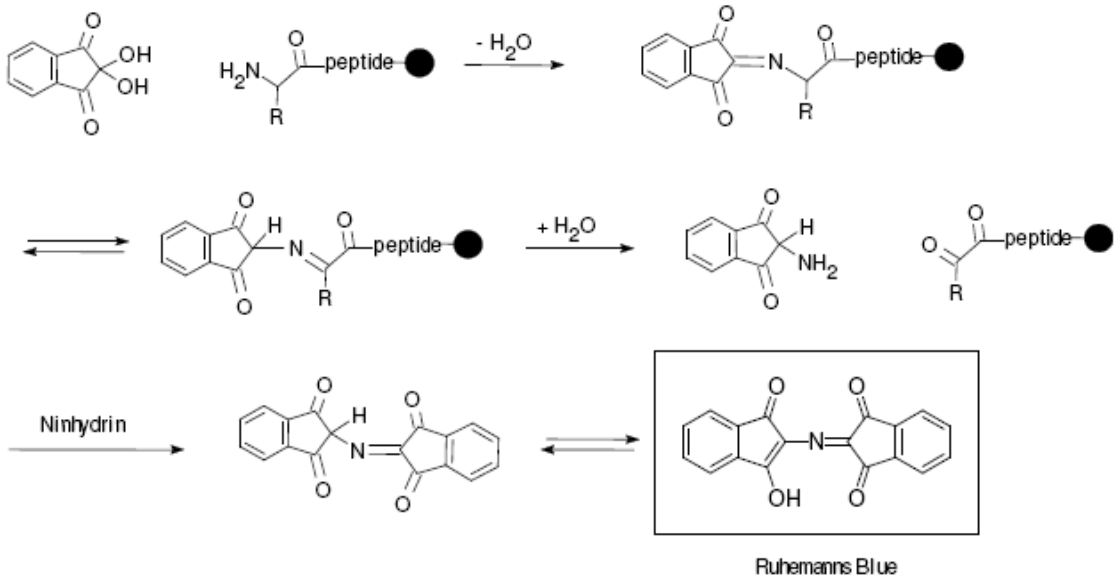
Step 3 (Colloidal Silica Solution)

1- Clean cloth surface with water and brush
2- Add colloidal silica solution to cloth surface.
3- Add solution continuously while polishing.
4- Polish for 3-5 minutes.
5- Wash cloth with water and brush extra material away.
6- Wash with soap water, rinse with water, and dry with air.
7- Examine surface at 4X magnification in the microscope.

Appendix C

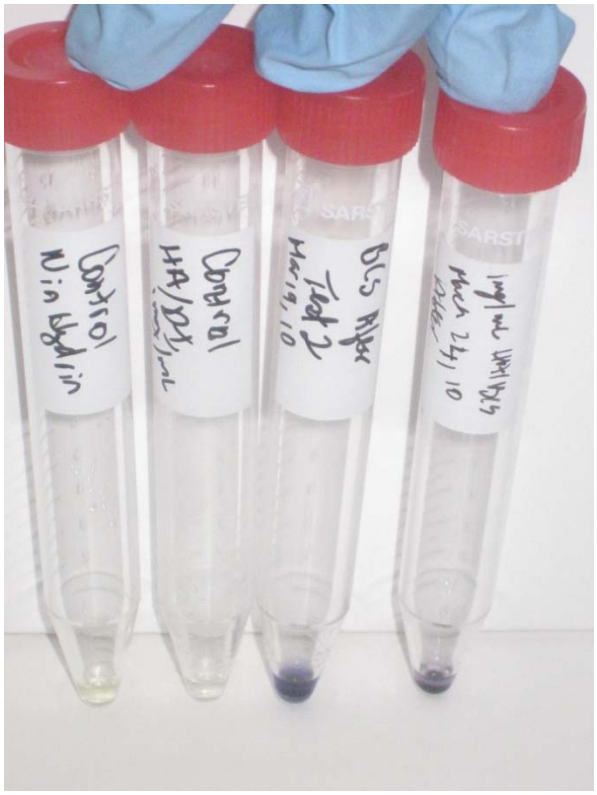
Ninhydrin Test Details and Results

C. 1 Reaction Scheme



C. 2 Test Results

C.2.1 BCS and 1mg/mL HA/BCS



C.2.2 Albumin and 1mg/mL HA/Albumin