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Rheological Characterization Of Nano-Composite Hydrogels

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Jack Lombardi

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in Partial Fulfillment of the

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Jack Lombardi

We, the dissertation committee for the above candidate for the

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Abstract

Engineered Polymer hydrogels and hydrogels from Bio macromolecules have visco-elastic properties that can be measured using Oscillatory Shear Rheology. Manipulation and measurement of physical properties in gels including F-127 Pluronic Block Co-Polymer and Poly(N-isopropylacrylamide)-Clay are shown through OSR by addition of salts, clays and glucose at physiological levels. Rheological analysis of f-127 illustrates changes in G' reduction with phase transition temperature. Measurements also indicate physical changes due to the aforementioned additives vary as a function of the gel physical and chemical structure. In particular, non-enzymatic glycation is shown to change the modulus of elasticity in both of the gels tested. Rheological analysis is also interpreted to produce a reduction In gel mesh size in the PNIPA –clay gels due to a possible co-solvency between phases of varying degrees of hydration.

This dissertation is dedicated to my wife Sharon. Without her support and understanding this work would not have been possible. Her encouragement after my hospitalization for obstructed heart arteries has been invaluable at a time in my life when I could have stopped and retired. She is my companion for 38 years of my life and It is my fervent hope that we can be together for another 38 years.

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I would like to thank my school colleagues who have helped me generate test plans, materials and provided camaraderie that was not present when I attended the Polytechnic Institute of N.Y. Brooklyn N.Y. Although I spent many years studying in Brooklyn for my Bachelors and Masters degrees, I feel strongly that Long Island and Stony Brook in particular is a special place. People tend to feel they belong and are willing to share resources and extend themselves. This thank you is extended to Jun Jiang, Divya Bhatnagar, DiXu and Liudi Zhang. Without their help and support this dissertation would not have been accomplished.

I would also like to thank my professors at Stony Brook who have helped me understand Polymer Science and gave me insight, information and training necessary to achieve the scientific learning and test results I needed to accomplish this thesis. Thanks to Prof. Koga for giving me knowledge of polymer physics, also thanks to Prof. Sokolov for his patience and understanding in providing the insight and review of physics and mathematics I needed after a rather long hiatus from those areas. I would also like to thank Prof. Halada for his kind support allowing me to use the surface analysis equipment in his lab when I needed it.

It is no secret that I work for a wonderful company, Estee Lauder, and there have been some very understanding and supportive people that I need to thank not only as supervisors but as colleagues and friends. In this regard I am referring to Dr. Nadine Pernodet, V.P. of Basic Research and Development and Dr. Thomas Mammone, Executive Directive of Skin Physiology and Pharmacology. Tom is my immediate supervisor and an extraordinary biologist who has actually encouraged me almost every day for the last 4 years to complete my PHD. He graciously agreed to be my fourth committee member which I am extremely proud of.

Finally I would like to acknowledge the support of my family members including my wife Sharon, my son Ross and my daughter Michelle for putting up with their dad . I would also like to thank my son in law Ragi for his encouragement. There were many weekends spent at work in the laboratory and up to 11PM weekdays that prevented me from being home with them. I would also like to thank the smallest but cutest member of our family my granddaughter, Alexa. To be honest she is probably my single biggest joy for the last 4 years of my life. How can someone so small make such an impact on my life?

x

Vita, Publications

> **Manuscripts first author**

Rheology of Poly (N-isopropylacrylamide)-Clay Nanocomposite
Jack Lombardi , Di Xu, Divya Bhatnagar , Dilip Gersape, Miriam H. Rafailovich
Jonathan C. Sokolov and Thomas Mammone: 2014

Rheology of Glycated Poly (N-isopropylacrylamide)-Clay Nanocomposite
Jack Lombardi , Divya Bhatnagar , Dilip Gersape, Miriam H. Rafailovich ,
Jonathan C. Sokolov: 2014

> **Publications Co-Author/Contributor**

Rheological Characterization of in Situ Cross-Linkable Hyaluronan Hydrogels K., Ghosh, J. Lombardi, Biomacromolecules,2005,Vol.6,Iss.5;p. 2857-2865

The effect of physiologically relevant additives on the rheological properties of concentrated Pluronic copolymer gels
J. Jiang, J. Lombardi, Polymer, 2008, vol. 49 p 3561–3567

> **Manuscript not yet submitted- first author**

Rheology of Porcine A Hydrogels Modified by Microbial Transglutaminase
Jack Lombardi, Luidi Zhang, Thomas Mammone , Miriam H. Rafailovich.
Jonathan C. Sokolov: 2014

> **Manuscript co-authored**

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Chapter 1

Introduction

1.1 Review of hydrogels tested and Rheological background

Generally gels are described as having a network structure that is bound by ionic, co-valent or in the case of pluronics by physical and ionic bonds as described later. Hydrogels are a classifcation of hydro colloids where the liquid phase is water. Their network structure is reversible when less strong ionic bonds dominate and is not reversible when co-valent bonds dominate. In the chapters that follow I have described gels consisting of all three aforementioned gel types and the means to evaluate them in terms of their physical response.

Hydrogels physical response consists of elastic and viscous shear components. The elastic component is recoverable and can be thought of as storing and releasing energy which can be described mechanically by a spring. The viscous component is non-recoverable and is described mechanically by a shock (like an automotive shock absorber). Combinations of these components constitue the visco-elastic response (Maxwell equivalent combination) which is separated during testing in an oscillatory shear experiment using a rheometer.

The hydrogels we chose to examine were made from aqueous solutions containing polymer materials: Pluronic 401 (F127) block co-polymer, PNIPA-clay (NC gels) and Porcine A gelatin powder (PAg). In all cases the visco-elastic response necessary to describe their behavior was provided primarily by OSR testing which is uniquely capable of separating and characterizing their visco-elastic properties . Chemical and structural details of each polymer hydrogel are described separately in the chapters herein based on structure and properties unique to each system. These hydrogels were chosen because we initially theorized that their sensitivity to solvent changes and addition of physiologically relevant additives such as salts, clays and proteins made them good candidates for documentation of mechanical properties using OSR techniques.

Since all of the hydrogel systems consist of solvent swelled polymers, it is only appropriate that a description of swell able polymers is in order and should include synthetic as well as biopolymers which were both examined . Insolubility (not solubility) in the solvent and the ability to organize the solvent in the surrounding space between the polymer and solvent is one explanation of sol-gels. hydrogels are a class of swell able polymers that can be described as those polymers that use water as a solvent. Our first investigation includes block co-polymers known in the industry as Pluronics. Hydrogels made with this triblock co-polymer; polyethylene oxide99–polypropylene oxide69–poly-ethylene oxide99 create physical gels which we will describe in some detail in this dissertation. Pluronic physical gels are unique in that they form micelles that organize from the dangling block strands of this linear polymer due to insolubility of one of the block strands (PPO). As packing builds from high concentration of polymer, these

micelles organize into semi-crystalline arrays and have been shown to be a single large lattice of FCC structure (1) Upon addition of salt, the lattice reconfigures into HCP (2).

Our next polymer hydrogel - PNIPA-Clay is formed from linking N-isopropylacrylamide monomer strands between clay platelets using Potassium Persulfate (KPS) as the initiator. KPS has more affinity for clay platelet "crosslinkers" than the monomer itself. The structure formed leaves us with a relatively stiff elastic material that has an inverted phase diagram allowing a phase separation at about 32C-34C. We have shown through molecular modelling of this system along with rheological review of stress sweep and frequency sweep data that a critical percolation concentration lies at around 15.24g/L clay. We are also able to show that this gel has sensitivity to glucose as a result of glucose effect on water. The chemical potential change from glucose bearing water acts like salt water and can affect the unfunctionalized PNIPA–clay gel in ways similar to other investigators that required co-polymerization or functionalization with water swelling moieties. (3,4).

Our final hydrogel system, Porcine A gelatin is made by denaturing collagen starting material from acid digesting pigskin, to dissociate the protein segments then subsequently heating to accomplish final denaturation (Ikada,2002). Material is then dried to produce a powder which consists of protein segments from the original collagen structure. On re-naturation with water, the powder, which contains the protein segments spontaneously forms helices that are not fibrous as was the original collagen matrix. This was verified in experiments by freeze drying prepared gels in Delbeccos Modified Eagles Medium and Distilled Water. The gels were examined by Scanning Electron Microscopy at high magnification of 100,000X. We found no evidence to support fibrous structure

and therefore conclude as others have that the gels consist of uniformly distributed helices containing organized water in and between protein segments along the spiral chain (5) .

Early descriptions of hydrogel materials, before they were fully described in modern technical terms, included ;swelling capacity, viscosity (not to be confused with a description of visco-elasticity) and phase diagrams derived from microscopic evaluation of heated and cooled samples. Although we still find these older parameters useful today, it is clear that a more sophisticated approach to gel description as well as characterization is required. This is especially important when engineered hydrogels are used. As an example for the uninitiated we can consider visco-elastic materials response for materials as common and different in physical response as Ketchup and Molasses. These materials are not overly complex and very familiar to all of us but viscosity alone is not adequate to characterize them and in fact they require separation of viscous and elastic response to do the characterization justice. Molasses which is highly viscous, does **not** have a yield point and so it flows over time under its own weight. A yield point describes the stress level which must be overcome before a visco-elastic material will start to flow inelastically or like a viscous liquid such as water or silicone fluid. Since molasses is known to flow without appreciable applied stress and can do so under its own weight, we say it lacks a yield point. Ketchup has a yield point which can be understood by its ability to resist flow out of a bottle unless we overcome the yield point with a good push or shake of the bottle.

Below the yield point it will not move but when the YP is exceeded.. well we all have experienced the mess it can make coming out of the bottle at relatively low viscosity and high flow. These different visco-elastic materials can be easily identified and documented with the advent of rheological techniques and the introduction of associated oscillatory shear rheology (OSR) equipment.

1.2 Goal of Research and reason for investigation

The overall goal of this research was to characterize nanocomposite hydrogels physical response using physiological solvents and additives with rheological test techniques particularly oscillatory shear rheology (OSR). Individually each of these polymer hydrogels has unique structure lending itself to a particular application which can be tuned using OSR analysis. For example: Pluronic block co-polymer F127 is liquid at temperatures below approximately 14C and gels above that temperature. At concentration of 30% by weight, its modulus is within the range found in spinal disc gel material (6) . It was therefore reasoned that we could repair spinal disks by injecting cold F127 into spinal discs which would fill the disc cavity and gel at body temperature. This would allow us to generate a replacement or repair system for spinal disc injuries. Later investigation by others improved on the material properties of F-127 alone by using clay and other additives to produce secondary stiffening of the gel J.Jiang et. al..

Using OSR techniques we found that PNIPA-Clay builds stiffness with increasing clay concentration following a power law relationship conforming to Mooney's equation.

(manuscript Rheology of PNIPA-Clay). This is surprising since Mooneys equation was developed from polymer melt investigations not polymer gels. At a specific concentration of clay (38.2g/L) which is beyond the percolation concentration of 15.24g/L clay found by our molecular model, our PNIPA-clay nanocomposite is sensitive to addition of glucose in the range of diabetic pathology (1-3mg/mL glucose). At this clay concentration its modulus of elasticity is reduced by a large amount (59%) and is associated with a reduction in polymer relaxation coefficient. We think this non-functionalized hydrogel when tuned properly as evidenced by our OSR analysis at various clay concentrations could be used for drug delivery including insulin delivery for diabetic patients.

PNIPA is of interest to many due to its lower critical solution temperature (7,8). This behavior produces a matrix swelling/contraction useful for drug delivery since the transition occurs below body temperature at around 32C. We found changes in G' (gel stiffness) can be generated if glucose is added as a solute to water in this hydrogel. Glucose has been reported to cluster water(9) and act to produce a secondary solvent which has the ability to modulate water attached to the hydrogel. We think this is the mechanism which creates the changes to shear modulus found during rheological analysis tracked by OSR in our PNIPA-Clay glucose paper.

1.3 Outline of Chapters

In chapter 2 we summarize the work performed on Pluronic F127 hydrogel polymers and the polymer structure. Strain sweeps were conducted using a Rheometrics Fluids Spectrometer II rheometer equipped with strain control motors. Where more

power was required an AR2000 T/A instruments rheometer equipped with dag-cup motor was used in stress control mode. Verification of polycrystalline gel structure was obtained using SANS intensity patterns from National Institute of Standards U. Minn. NG3 beam line and SAXS was performed at the X3A2 SUNY beam line. DSC results were used to corroborate transition temperature obtained by OSR measurements which were obtained using a temperature programmable Peltier plate on the rheometer. Effects of added salts, clays and glucose are summarized and presented to illustrate the extent of each additive on physical properties and the consequence of applications for this polymer.

In chapter 3 we present the rheology of PNIPA-clay gels which were made free radical polymerization with clay. As described repeatedly by Hariguchi and others (11,12), the clay plates were activated using an initiator having greater affinity for the clay surface than the monomer, PNIPA. This allows attachments of PNIPA to clay surfaces instead of monomer to monomer attachments. Since internal structure is an important basis for material analysis and we were curious as to the nature of the nanocomposite bond system. To pursue this , we generated a molecular model that made assumptions of the size and shape of Clay particles and the bonded strands of PNIPA monomer attached to the particles . The simulations, using LAAMPS program(13) were run to predict the percolation threshold of particle generation by attachment of the monomer to clay while the number and spacial arrangement builds in the solvent(14). While the system is building, a theoretical stress was applied to the matrix using stress autocorrelation function to test its reaction to stress (stress auto correlation function-

SAF) as the system builds and becomes populated with greater numbers and sizes of particles binding to clay. Based on SAF data we saw that as simulations were run at different clay concentration , the SAC function changes. At a critical clay amount, an abrupt change in SAC occurs which corresponds well with rheological (OSR) data at a particular clay concentration of about 15.24g/L.   At this concentration rheological measurements showed a non-linear increase in modulus with clay composition and a change from liquid dominated behavior to classical visco-elastic gel behavior (15). The Model therefore predicted the percolation clay concentration near 15.24g/L clay very well.

In chapter 4 we decided to pursue the property analysis of PNIPA clay further and examine the effect of addition of glucose on PNIPA-clay gels in a concentration range of 0-10mg/mL which is within the range of diabetic pathology(16). Some authors have shown that addition of glucose sensing moieties to PNIPA-clay composites resulted in gel systems capable of drug delivery including complex with block polymer or phenyboronic acid with NIPA as a system for delivery of insulin (17,18). We did not think these complexing molecules were necessary and it is known that they complicate the material manufacture. Based on our previous findings of NIPA-Clay rheological characterization we used a concentration range of clay that bracketed the range of percolation concentration (15.24g/L to 38.2g/L clay). This allowed us to see the effect of gel stiffness and cross linking on PNIPA-clay gel glycation directly. We found that below the percolation amount of clay (15.24g/L) G' and G'' are very low in value and unstable with respect to glucose addition. The yield values were so low that there is some question

as to whether they really qualify as gels at all. Since there was presence of a yield point we do consider them to be gels. As we transitioned to higher clay concentrations of 22.86g/L and 38.2g/L the gel became stronger, G' values became more consistent (values did not vary as much) and the effect of glucose was greater. At a concentration of 38.2g/L clay gels showed a 59% drop in modulus from 2mg/mL to 3mg/mL glucose. This magnitude and extent of G' change was greater than other gel clay concentrations leading us to conclude that OSR testing is necessary to determine the correct composition for greatest effect of glucose in this unmodified gel system.

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Chapter 2

Rheological Characterization of Pluronic Polymer Hydrogels Including Effects of Addition of Salts, Clays and Glucose

2.1 Introduction

F-127 Triblock polymer gels produce amphiphilic structure and therefore have the ability to carry hydrophilic and hydrophobic materials. This makes them an excellent choice for active materials that are either water or oil soluble. Cosmetic actives are difficult to dissolve in colloidal emulsions and create low PH environment which can be troublesome for stability but would be easily carried by Pluronic gels (1,2). These gels at 30% by weight in water as stated earlier are also liquid at low temperature (4C) and form stiff elastic gels above approximately 14C which makes them suitable as injectable drug carriers for controlled release where they can be placed in the area required by injection (or patches for dermal surface application) then allowed to gel and produce a timed release (1-4). The possibilities for use of this material are limited only be the ingenuity of the bioengineer who takes advantage of the special properties.

To make F127 gels we simply dissolved 30% by weight F-127 powder in water (looks like oatmeal at this point) then cooled the mixture overnight at -4C which is below the temperature at which Polyethylene oxide is insoluble in water making both block segments soluble in water resulting in the generation of a clear homogeneous liquid. The explanation for this phenomenon is that on heating, the PPO block becomes insoluble in water. We say the material has a lower critical solution temperature.This

forces the hydrophobic PPO block inward away from the water and the hydrophilic PEO

blocks outward toward the water forming a micelle gel (3-6).
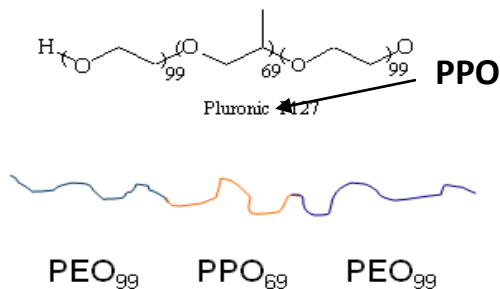
Figure 2.1a Pluronic F-127 block arrangement   Figure 2.1b F-127 micelle in water
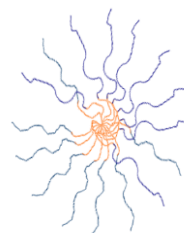
The resulting individual micelles configure themselves into spatially oriented spherical

particles in water to form what is known as a physical micellular gel.

Figure 2.1c F-127 Oriented Micelles (FCC structure)

The micelles are colloidally suspended until their concentration reaches the percolation

point at which the gel is formed. This micelle gel is ordered as shown in figure 1c above

(7). A description of the sol-gel transition is below in figure 2.

Figure 2.1.2 Sol-Gel Transition

The sol-gel transition occurs when enough micellular particles connect to form the large structure considered to be the polymeric stable gel. This particle system has "percolated" into the stable ge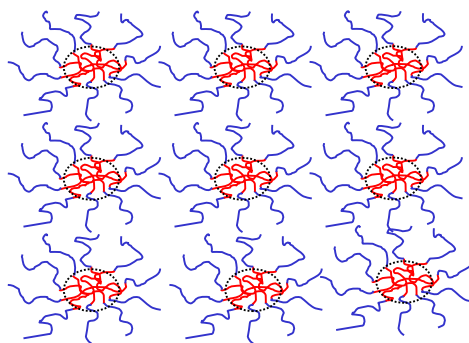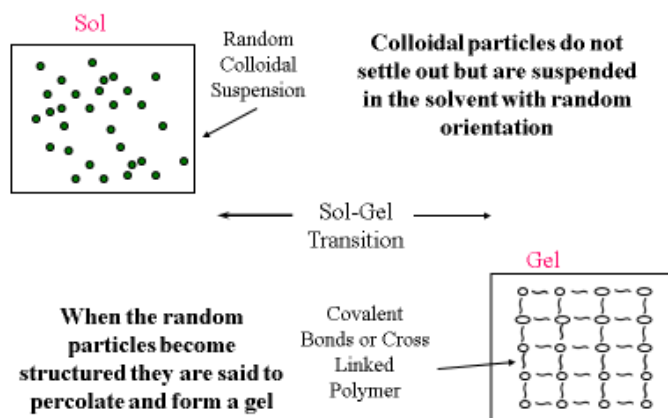l state from the colloidal random particle state. This is called the sol-gel transition. We also recognize this as critical to our molecular model in later discussion of N(Isopropylacrylamide), a monomer which is cross linked with clay by free radical polymerization(7). That model shows the percolation transition at a low clay concentration evidenced by change in matrix stiffness and relaxation time before and after percolation.

2.2 Experimental

The resultant F127 gel that occurs on heating from the liquid state at -4C to room temperature (25C) exhibits visco-elastic response (8) which we characterized by OSR technique using primarily cone-plate geometry. OSR can also be performed while the mixture transitions from the liquid to solid state using concentric cylinder (CC) or double

concentric cylinder (DCC) geometry which confines the liquid in a manner similar to simple Brookfield viscosity testing in a jar.

Brookfield Spring Driven Manual Viscometer

This is the most common viscometer in the world

Figure 2.1.3

The main difference between OSR and Brookfield viscosity test is that the Brookfield technique(9) actually only measures viscosity at one steady state shear from rotation of the moving rod or cylinder in one direction at one particular rpm while OSR technique
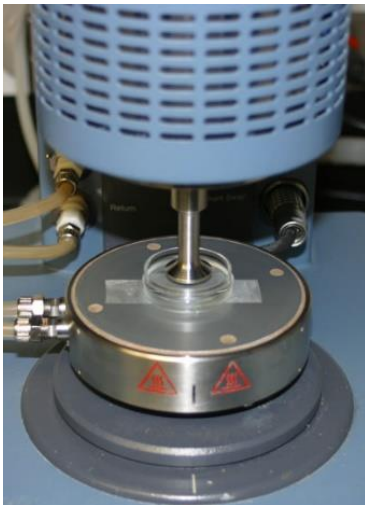
Figure 2.1.4a -2cm cone-plate (AR2000)

Figure 2.1.4b- Concentric Cylinder (AR2000)

is usually used in oscillation mode where we alternate the moving cone or plate (or bob/cylinder for Concentric Cylinder or Double Concentric Cylinder geometry) in the clockwise and counter clockwise directions (from reference zero position) during measurement (Figures 3a and 3b). The motion produced is recorded and compared to the motion programmed so that if there is a difference it can be analyzed. Rheometers

continuously vary the applied torque from a drag-cup motor which has low inertia so that

compensation for inertia is minimal and we obtain position information from a degree

wheel with optical sensor and encoder using an inline continuous shaft connecting both

components(10) . Drag-cup motors have open cup like rotor designs that reduce weight

and therefore reduce inertia culminating in an increased measurement sensitivity. The

drag cup rotor is not solid but hollow in the middle shaped like a cup and usually attached

at one end. Torque is developed by stationary coils which magnetically couple to the

rotating cup. To supply position information, a degree wheel is mounted in-line with the

motor shaft placed at one end of the shaft and the instrument has optical sensors which

provide rotational position information vs. time which are usually at the other end of the

shaft. Both components are designed to interface with analog to digital converters and

other electronics that are capable of separating the phase relationship between applied

torque by the rheometer and induced movement by the sample. When a sample which is

placed in the geometry gap and the rotor connected to the motor are completely coupled

this phase relation is zero, G' dominates and the system represents an elastic solid. When

the sample is completely uncoupled it is (at least) 90 degrees out of phase and represents

a viscous liquid. This phase angle, tan δ is used to separate viscous and elastic

components of sample response as opposed to the instrument programmed wave form

locked, specified and made by the operator. This system constitutes the unique modern

technology for measuring visco-elastic response of materials. In the below illustrations

describing OSR techniques, the motor provides torque that generates stress based on

sample geometry and the sample moves (strains) in response to this torque. The red lines

represent motor information and the blue lines represent sample information. Tan

δ=G"/G' Where the angle between sample and motor response curves is δ.

Liquid Like (G")   stress
strain

90°   δ

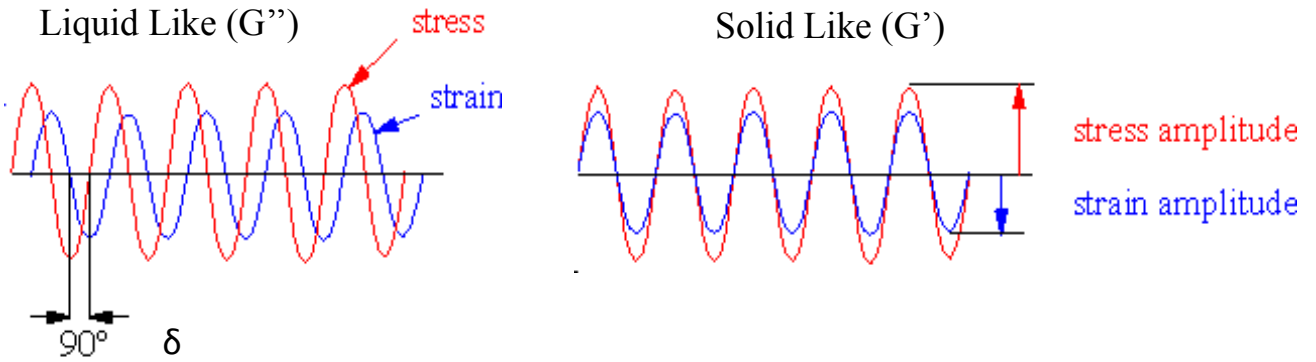Solid Like (G')

stress amplitude

strain amplitude

Figure 2.2a Liquid like sample response   Figure 2.2b Solid like sample response

   When the effect of temperature on visco-elastic properties of F-127 is desired, a

programmable temperature controlled peltier plate is used which can progressively

increase temperature from the solution state of F-127 which is 4C to the gelled state of

14C at increments as low as 0.1C. By doing so we can find the sol-gel transition region

as shown by our data using the CC (concentric cylinder) geometry. In this geometry we

must start from the low temperature liquid state below the gel transition. In this case the

sample can be added by filling the cylinder with F127 liquid at 0C and immersing the bob

or concentric cylinder to a predetermined position prior to test. The peltier plate is then

ramped up in temperature as the rheometer cycles and records the sample response.

        Sol-gel temperature transition T_t can also be found by starting with gelled

material using a plate-plate system at a temperature above 14C (visco-elastic state) using

a flat plate geometry. The sample is slowly compressed between the stage and flat plate

geometry before testing. The temperature is then decreased during testing from gel to

liquid state during oscillation to find the gel to liquid temperature transition by observing

the stress strain relation produced. F-127 physical properties transition is reversible so

either the plate or cylinder method can be used. Usually, plate or cone geometries are

limited to gels and emulsions which have elastic response and/or yield point due to the

unsupported space at the edges of these geometries otherwise the sample will flow out

and change the sample volume during test. CC geometries are able to contain the sample

edges within the cylinder so that use of the rotating component (bob or second internal

rotating cylinder) is not affected by loss of material however sample introduction must

be initially by liquid to fill the cylinder. In both types of geometry CC or plate-plate (or

cone-plate), OSR tests results show that the technique is sensitive enough to provide an

indication of how changes to the solvent used by addition of salts, protein or glucose can

affect physical properties.

2.3 Effect of Additives

Behavior of f-127 with dilute salt and clay concentrations was analyzed by others

at low concentration(11-15). We analyzed addition of protein , salts and glucose at

relatively high concentration which until now was only studied in relatively few instances

(16-17).
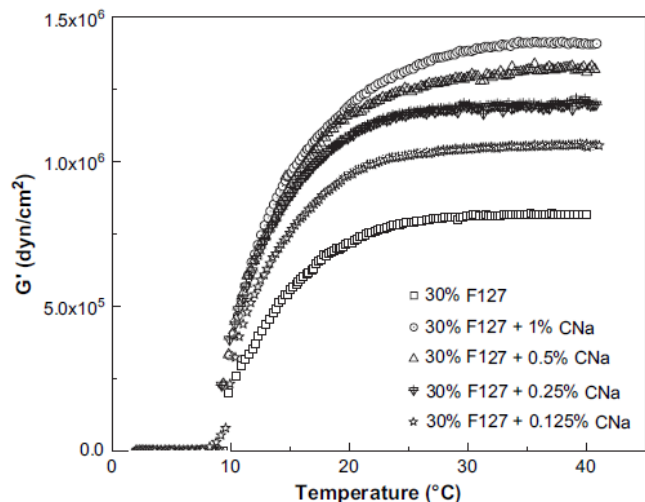
Figure 2.3a Cloisite clay effect on T_t transition temperature
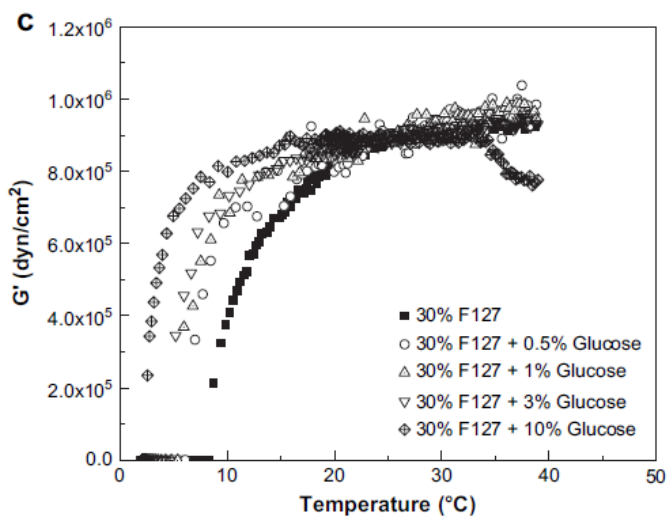
Figure 2.3b Glucose effect on transition temperature
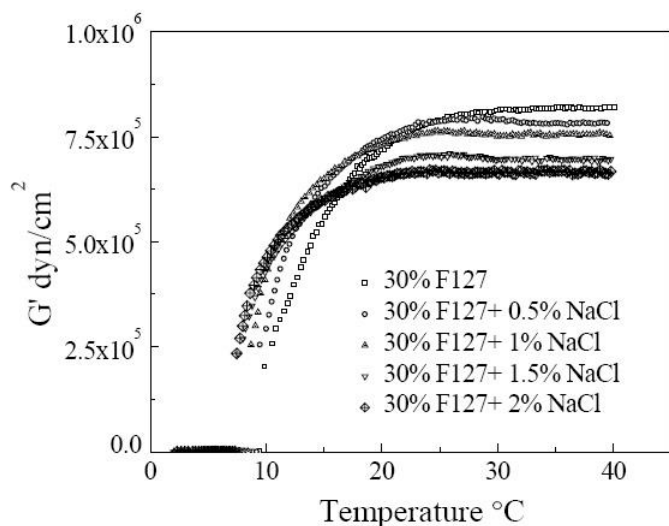
Figure 2.3c NaCl Salt effect on T_t transition temperature
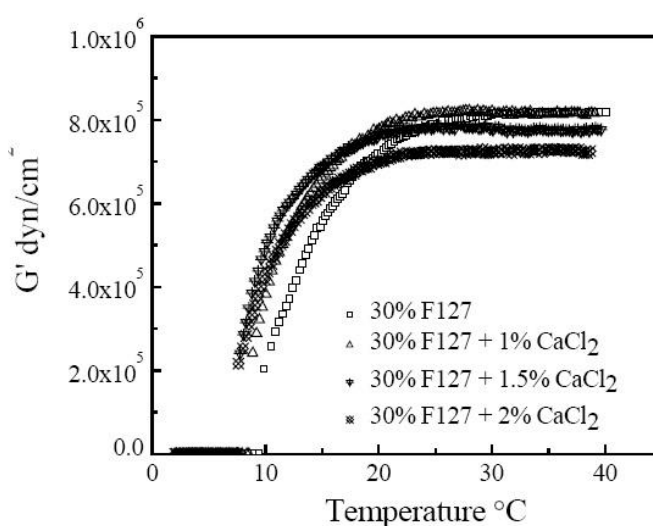
Figure 2.3d CaCl Salt effect on T_t transition temperature

We determined by OSR that clays (figures 2a,2b,2c and 2d) and salts affected the transition temperature and resulting physical properties of the gel. We also saw that F127 transition temperature was influenced by addition of glucose (figure 2.3.e) as much or more than changes from clay (Figures 2.3.a, 2.3.b). This was unexpected. Glucose did not ,however, appreciatively lower G' as it did in other gels such as PNIPA-Clay.
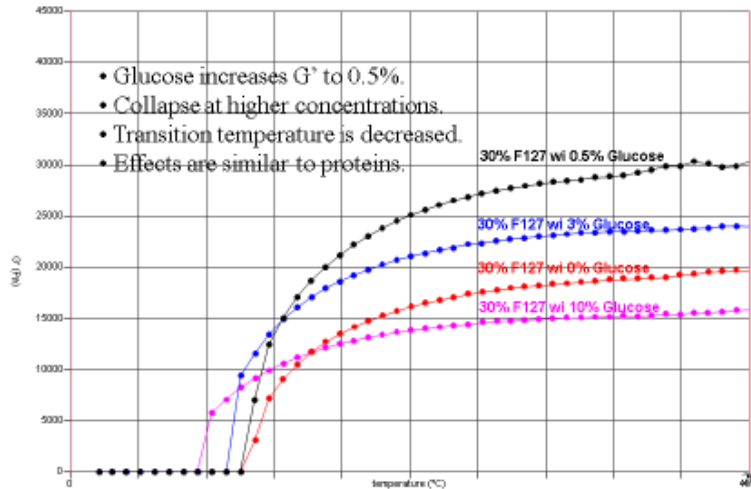
Figure 2.3e F-127 response to glucose

2.4 Conclusions

This physical micellular gel material has a configuration which has many advantages over conventional polymer hydrogels. It can be easily made by dissolving a high concentration of the polymer into water and after cooling to 4C it becomes a clear fully dissolved liquid solution at concentrations as high as 30% F127 by weight. After heating the dissolved solution above 14C, F-127's gel system is quite rigid having a modulus in the order of several hundred thousand dyne/cm2 (~800Kdyne/cm2) which is within the range of gel material of human Nucleaus pulposis spinal disc material (18). This means it could be used to inject replacement gel into spinal discs to accomplish their repair. This block polymer forms an amphiphilic gel capable of carrying hydrophobic materials in the nucleus of the micelle or hydrophilic molecules in the outer water corona which makes it unique as a carrier for cosmetic drug application on skin surface especially for hard to use molecules as Salicylic acid with low PH and low water solubility.

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F127 hydrogels semi crystalline structure has high rigidity that is compatible with nucleus pulposis gel in human spinal discs. An interesting physical property of this gel system is that it is a liquid below approximately 14C due to the solubility of PPO in water at low temperatures. Above the solubility in water of the PPO block the highly soluble PEO blocks generate a corona around the PPO block creating the physical micelle. At high enough concentration the micelles arrange into a crystalline lattice structure which is FCC until disturbed by exterior stress which transforms them into HCP. The gels are ionically bound so they are completely reversible and can change from liquid to gel and back without degradation.

2.5 Supplemental Information

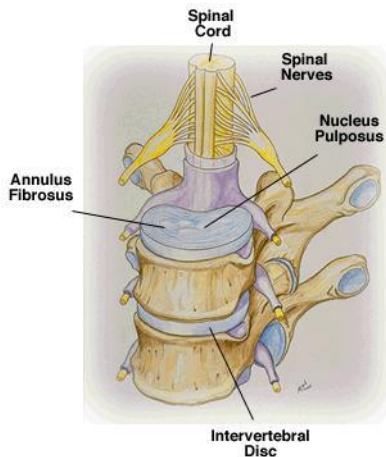

Studies have shown that Human Nucleus pulposis has a strength in the order of 7.4–11.3 kPa equal to 74,000-113,000 dyne/cm2

Our F127 system had 300,000 dyne/cm2-800,000 dyne/cm2

Figure 2.6f Spinal Chord segment

Preliminary work in canines on herniated lumbar discs was successful. The system was tested in collaboration with Dr. Ralph Davis Chair of Neurosurgery (Stony Brook Medical Center).

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Chapter 3.0

Rheology of Poly(N-isopropylacrylamide)-Clay

Nanocomposite Hydrogels

3.1ABSTRACT:

We used Molecular Dynamics simulations and experiments to study the rheology of polymer-clay nanocomposite hydrogels. The Molecular dynamic simulations studied the formation of physical networks as a function of the clay concentration. Simulations showed that while the local structure changed from isolated polymer-clay clusters to a percolating network with increasing clay concentration, the networks were only able to sustain stress at concentrations of roughly 1.5 times the percolation transition. Experiments using poly(*N*-isopropylacrylamide) (PNIPA)-clay nanocomposites at different clay concentrations were compared to simulation results.

Transition from G" dominated to G' dominated were observed for composites at clay concentration 15.24 g/L, which indicate the change from viscous fluid to gel, in good agreement with the simulations. The yield modulus, G' and G" was observed to increase

exponentially with clay concentration. This exponential behavior was similar to what has been observed in polymer nanocomposites and could be fitted to the Mooney equation.

3.2 Introduction

Polyacrylamide (PA) has long been known for its ability to swell in water to make soft clear gels. Since the discovery by Heskins and Guillet in 1968 that PNIPA, a variant of PA, exhibits a lower critical solution temperature attributed to a coil to globule transition,[1,2] study of this material intensified.[3-5] Unfortunately it was found to exhibit relatively brittle behavior compared to its PA predecessor, thus limiting its use for biological and industrial applications. Haraguchi and others[6,7] later demonstrated that it was possible to initiate free radical polymerization of NIPA from the clay surface, thus making a strong nanocomposite (NC) hydrogel which was thermally responsive within a physiological temperature range. This enabled numerous applications for PNIPA, such as: drug delivery systems,[8,9] rapid release cell culture substrates,[10,11] flocculation additives,[12] separation devices[13] and wound healing dressings.[14]

The specific applications chosen for the PNIPA-clay gels have also necessitated fine tuning the mechanical properties, which was found possible via changes in clay concentration[6] and solvent quality.[4] However, earlier studies of polymer-clay gels have focused on regimes above the gelation point (or didn't explicitly distinguish gelation threshold). We note that in earlier studies the mechanics were mostly tested using strain or compression techniques,[1,2,10] that do not provide sufficient rheological information subjected to shear forces. Further, the subtle mechanical changes and structure at the gelation point have not been studied. Such studies will yield valuable information into the formation of these networks and the factors that control their formation. Our goal in this

paper is to use theoretical studies to examine the onset of gelation in PNIPA-clay gels and the mechanisms that control the properties of these gels. We then plan to initiate a series of experimental studies probing the rheology of these gels, and also to develop a theoretical framework in order to characterize the rheological response of these gels.

In this paper, we use both theoretical and experimental methods to understand the fundamental phenomena that govern the properties of PNIPA-clay hydrogels. Molecular dynamics simulations and Mooney's theory have been used as means to analyze the onset of gelation and the mechanics of the gel. These are combined with a set of rheology measurements on PNIPA-clay nanocomposites, where we use a range of clay concentrations, from clay-polymer solutions to very stiff hydrogels. The large amount of data characterizing the PNIPA-clay nanocomposites and the molecular details from the simulations make this system an excellent platform for the development of new NC gels with their rheological response tailored for specific applications.

3.3 Molecular Modeling

Molecular Dynamics (MD) model is an ideal tool to study molecular structure and dynamics of gels. Xu et al [35] developed a model to explain the data presented here where the clay particles were approximated as small rigid bodies, which could physically bond to the polymer by a Lenard-Jones (LJ) force. This enabled them to simulate a large enough system to look at the dynamics of global network formations. The theoretical model was able to explain two major results of the experimental work;

(a) We studied the effect of using isolated spherical nanoparticles and compared these to our platelets. The aim here was to understand the role that the aspect ratio of the nanofiller had on the properties of the gel. We simulated systems with 2.15% volume fraction of fillers,

either in the form of spherical particles of diameter 1.0σ or sheet platelets described above. The simulations varying the shape of the particles indicated that the platelet or sheet structures could produce much stronger gels than spheres as shown in figure 3.3.1 indicating the shapes of fillers and figure 3.3.2 indicating the stress corresponding autocorrelation values for sheets and spheres.
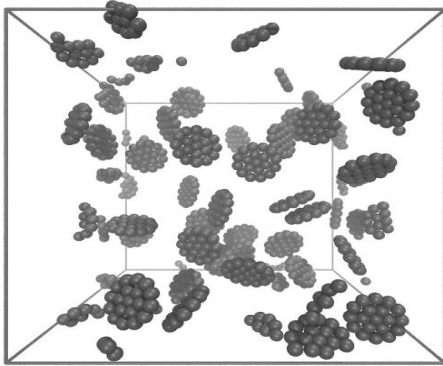
Figure 3.3.1 Simulation particle shapes
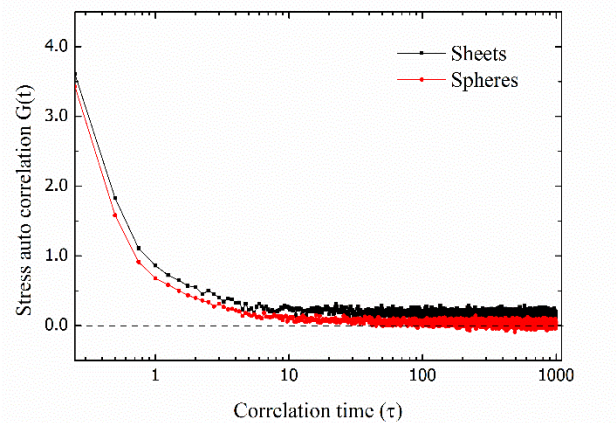
Figure 3.3.2 Simulation response is more for sheets than for sphere

(b)   Calculating the autocorrelations between particle/filler junctions, they were able to simulate network formation and determine the point at which percolation occurs and the network becomes mechanically stable. The results are plotted in figure 3.3 where they show that a transition from a viscous response to an elastic response, occurred at clay volume fraction 2.15% is roughly 1.5 times greater than the percolation threshold of 1.45%, that we observed in our system.
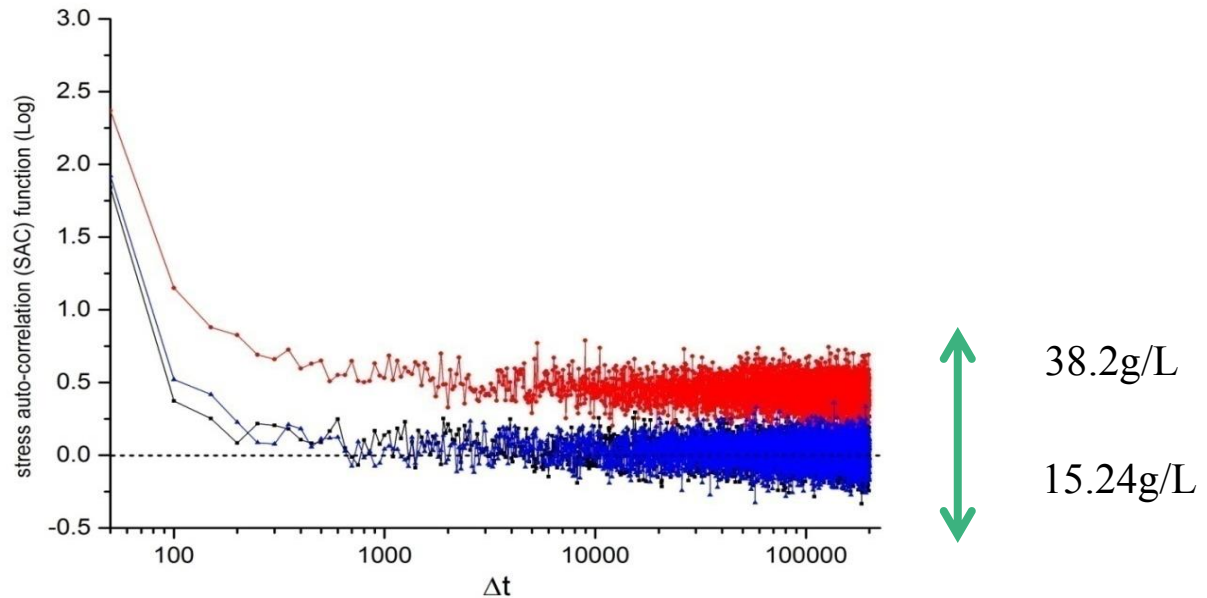
Figure 3.3.3 Stress auto correlation function of simulated composites at correlation time for two different clay concentrations as illustrated to the right were 15.24g/L clay and 38.2g/L clay

It should be pointed out that when clay concentration goes past the critical gelation point, our simulated system became non-ergodic (or extremely slow dynamics that hardly equilibrated in simulation); we observed SAF and stress relaxation tails show large variances between different initial conformations as the clay fraction goes above 2.15%. Simulation data of higher clay concentrations were not presented to avoid over-interpretation, but in all cases, above the transition point, SAF and stress relaxation curves all have a non-zero tail, and become more significant with increased clay volume fraction.

3.4 Experimental

3.4.1Materials. *N*-isopropylacrylamide (95% purity) was from Sigma-Aldrich (St. Louis, Mo). Deionized water was obtained from a Barnstead E-Pure 3 stage water purification system with conductivity of 18.4 Meg-Ohms minimum. Residual oxygen was removed from the pure water by bubbling N_2 gas into water for 5 minutes prior to addition of ingredients. Synthetic hectorite laponite XLG was obtained from Rockwood Specialties Inc (Chester, NY) with bulk density 1000 kg/m^3 and cation exchange capacity 104 mequiv/100g. Clay and NIPAs were grinded in mortar-pestle to obtain fine particles. Potassium peroxodisulfate (KPS) and *N,N,N',N'*-tetramethylenediamine (TEMED), initiator and catalyst respectively, were obtained from Fisher Scientific (Fairlawn, NJ).

3.4.2 Synthesis of PNIPA-clay gels.

In making all samples we used 50 mL water, 5.65 g NIPAs (1 mol/L), 50 mg KPS and 40 μL TEMED constantly. The clay concentration varies from 7.62 g/L to 190.5 g/L. For samples with clay concentration below 60.96 g/L, solids were mixed with water using a 5/8" turbine blade mixer at approximately 15,000 rpm. An ice-water bath was used to keep samples cool and below the lower critical solution temperature (LCST).[13,28] This also allowed us to keep foaming to a minimum. Samples were refrigerated after mixing Clay and NIPA to cool them, then again after addition of KPS and lastly after addition of the TEMED with or without glucose. For samples with clay concentration 60.96 g/L and above, a planetary centrifugal mixer was used for mixing.

3..4.3 Measurements.

Rheological samples for each test point were generated by pipetting 2 mL of liquid into 37 mm petri dishes. Samples were then allowed to polymerize for a minimum of 24 hours

in a covered glass bell jar. Rheological testing was performed using a Bohlin HR-Nano Rheometer (E. Brunswick, NJ), which has 1 nN·m (nano newton meter) torque resolution and 50 nano radian position resolution. These specifications are well below the lowest measured torque of 2.04E-7N·m and the smallest measured position displacement of 4.4E-4 radians. Shear stress sweeps were performed using 2.0 cm plate geometry in contact with the gel surface inside the petri dishes. A normal (contact) force of 10.0 g was used to eliminate slipping. All samples were tested at constant temperature 25.0 °C (which is below the reported LCST of 32.0-33.1 °C)[5,28] and constant frequency of 1.0 Hz, while the shear stress was ramped from low to high until the NC gels went beyond the elastic stress range and yielded. NC gels with clay concentration below 60.96 g/L were tested between 0.1 to 1000.0 Pa, and those at or above 60.96 g/L were tested between 10.0 to 10,000.0 Pa. An interval of 3 seconds dwell was imposed between oscillations to equilibrate the modulus.

3.5 Results and Discussion

At clay concentration of 15.24 g/L or lower, G″ is relatively low and initially remains relatively constant below the yield region (figures 3.4a). G′ drops considerably after yield point (yield stress about 2.9 Pa) and becoming lower than G". In these samples of clay concentration 15.24 g/L or lower, we observe that G" dominates the behavior throughout a considerable portion of the applied stress range. When the clay concentration is increased to 22.86 g/L (figure 3.4b) we see that G' is higher than G" at all stress levels prior to yield point (yield stress about 33.6 Pa). After yield there is a sharply defined crossover region of G' and G" which is classical rheological behavior for cross-linked polymers. The change from G" dominated to G' dominated behavior indicates that a transition occurs from viscous

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liquid like material to a "visco-elastic solid" gel state.[29] With increasing clay concentration both G' and G" continue to increase but the rate of increase of G' is always larger than G" so that the gap between G' and G" becomes more significant (figure 3.4c,d). This confirms that a critical clay concentration has been exceeded above which an increasingly solid-like (highly elastic) behavior is indicated.
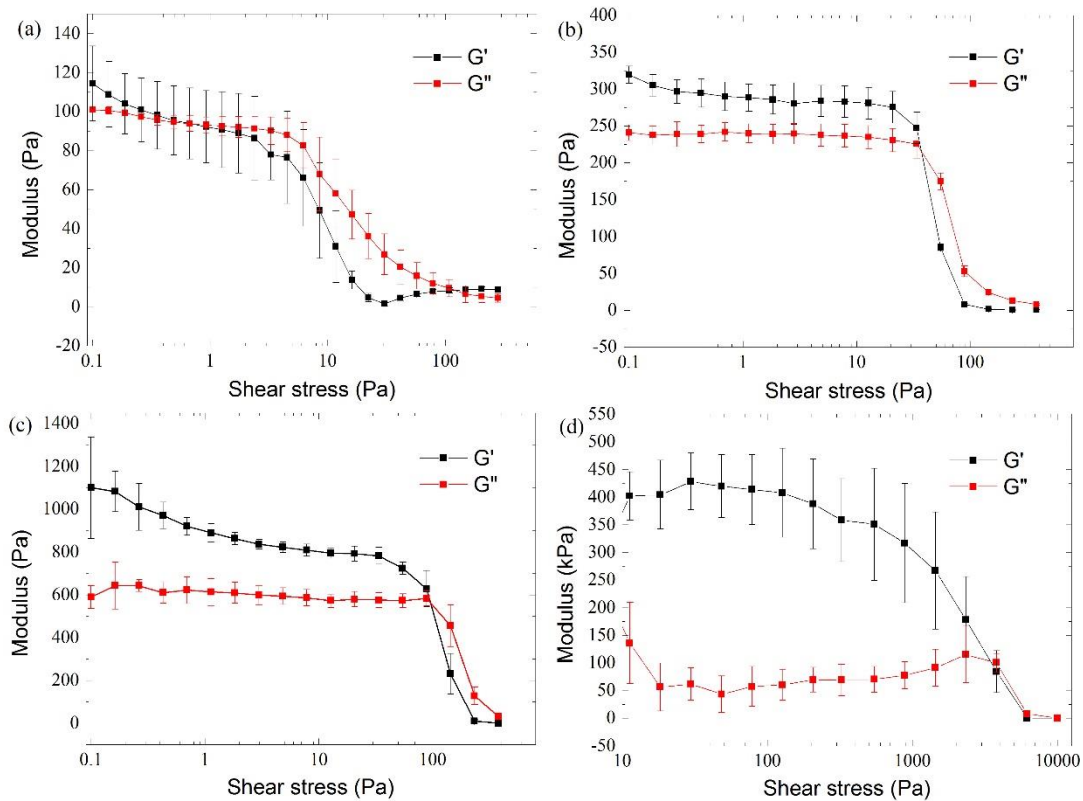
Figure 3.4. G' (square) and G" (circle) at different shear stress with clay concentration 15.24 g/L, 22.86 g/L, 38.10 g/L and 190.5 g/L, from (a) to (d) respectively. (Note the unit in (d) is kPa)

Both G' and G" moduli were found to increase significantly with increasing clay concentration. G' increased to several hundred kilopascals at a clay concentration of 190.5 g/L (yield stress about 2,336.0 Pa), which indicates a very stiff elastic gel. Closer

analysis revealed that the modulus of our PNIPA-clay gels increased exponentially with clay concentration. In Figure 3.5, we plot G' and G" obtained by averaging the values in the plateau region, versus clay concentration. From the figure we can see that Both G' and G" can be fitted on a semi-log plot with a linear relationship clearly indicating an exponential increase with clay concentration (as seen in Figure 3.5). We can also see that the rate of increase for G' is significantly larger than that for G", which is consistent with an increasingly solid like response.
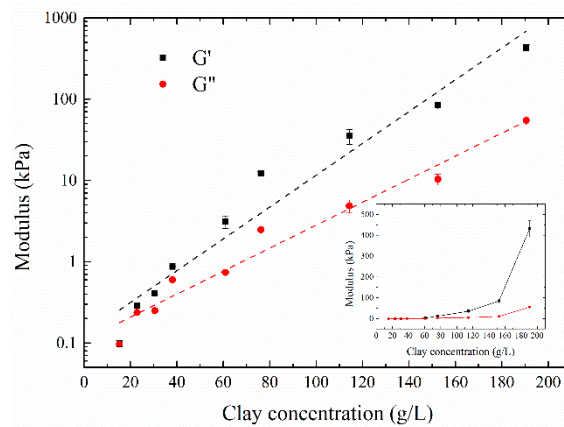
Figure 3.5 G' (square) and G" (circle) of PNIPA-clay composites at different clay concentrations (above gelation threshold concentration). The inset picture shows the original shapes of the curves and dashed lines were used to guide the eye.

Most polymer network theories predict that the modulus has a linear dependence on the network strands density, we think these theories hold for PNIPA-clay networks[30,31] but the exponential increase in mechanical properties could originate from the large aspect ratio of clay particles. Clays act as cross-linker in our system and at critical conditions it forms a gel, but even in the gel state, there are many dangling polymer chains that don't bridge between platelets. With increased number of clay platelets, we think that the number of dangling polymers are significantly reduced and the distance between cross-

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linkers decreases significantly.[31] Here the traditional picture of a cross-linker as a point junction is no longer valid as the effect of the cross-linker is spread over a much larger area. Indeed, our system appears to behave mechanically more similar to polymer melts than polymer gels at higher clay concentrations, where the collective motion of the gel extends over longer length scales.

Our PNIPA-clay gels are very similar to other nanofilled polymer composites which show that polymer-filler interactions significantly change composite properties. We noticed that Rao, et al. observed the Yonug's modulus of latex(PBSMaSO$_3$)-montmorillonite clay nanocomposite thin film exponentially increased with clay concentration and the results fitted well to Mooney's equation, similar method was applied to our G' values at yielding (calculated as on-set of dramatic change in slope downward) of PNIPA-clay gels.

Mooney extended Einstein's viscosity theory of dilute sphere suspensions to higher concentration by using a hypothetical crowding factor to account for pair-wise interactions. It could be extended to non-spherical particles by using a modified Einstein's constant,[32]

$$K_E = 2.5 * (L/t)^{0.645} \tag{1}$$

In Equation 5, K_E is the modified Einstein's constant as a function of filler's length L and thickness t. When Gy' (G' at yield point) is compared instead of viscosity, it yields the final form of Mooney's equation we used:

$$\ln \frac{G_y{'}}{G_{y0}{'}} = \frac{K_E \phi}{1 - k\phi} \tag{2}$$

In Equation 2, ϕ is the volume fraction of clays, k is the crowding factor between polymer matrix and added clays, as indicated by Mooney,[33] k is a function of filler and matrix particles size with theoretical range 1.35 to 1.91 for spheres, and G_{y0}' is the G_y' value of the matrix state. Assumptions have to be made to fit our results into Equation 1: We used k = 1.35 assuming that sheets could have dense packing; The volume fraction of clays was estimated from the weight concentration (dividing by it's bulk density 1000 kg/m^3), and we select our sample at critical gelation clay concentration of 15.24 g/L as the reference matrix state. With these assumptions, least square fitting yields K_E = 35.70 ($\pm$ 2.18), which is of the same order of magnitude as that calculated independently in Equation 5 using the dimension of the clay particles that has diameter 30.0 nm and thickness 1.0 nm.[10] The fitted curve is shown in Figure 3.6. This result is very surprising and illustrates that the modified Mooney relationship applied for static modulus of polymer nanocomposite[32,34] is also applicable to the sheer modulus of a polymer hydrogel.
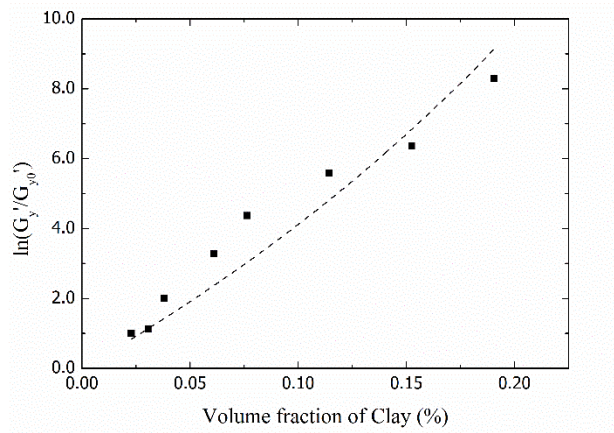

Figure 3.6 ln(G_y'/G_{y0}') at different clay volume fractions (above gelation), the dashed line is fitted to modified Mooney's equation (Equation 1).

3.6. Conclusions

Our studies of PNIPA-clay composite hydrogels allowed us to correspondingly indicate that mechanics were strongly dependent on clay concentrations. Simulations showed that although the percolation transition occurs at lower clay concentrations, the mechanics of the system reveal that the gel transition occurs at roughly 1.5 times the percolation transition. Simulations also showed that the aspect ratio of the filler is critical to forming a gel. Spherical nanoparticles (at the same volume fraction) did not result in a gel. This is evident even though the interaction between the polymer and the nanoparticles were kept fixed, and though the spheres offered much more surface area for interactions. We believe that the aspect ratio of the platelet allows for multiple chains to adsorb (on both sides) creating a much stronger cross-linking junction. We confirmed the simulation data by performing rheological experiments on a PNIPA gel with clay nanoparticles. Experimental data suggested a solution-gel transition at clay concentration about 15.24 g/L. where G' levels increase by an order of magnitude and dominated compared to G". We note that if we convert this weight fraction into a volume fraction (by using bulk density of clays, 1000 kg/m^3) we get a volume fraction that is comparable with the simulation results for the gelation transition. We realize that our estimation of the volume fraction in experiments is an approximation, but nonetheless, it is in the same range as the simulations predict.

We found the modulus, G', G", and Yield modulus, increased with clay concentration exponentially, as a result very stiff gels were achieved at high clay concentration. This

trend of exponential increasing was believed due to the large aspect ratio of clays, which caused more than a simple linear increase with cross-linker density. Our G' results surprisingly fit a modified Mooney's equation, which was proposed for viscosity of sphere suspensions, in low to intermediate clay concentration.

3.7 Supporting Information: Full range of PNIPA-Clay Rheological Tests
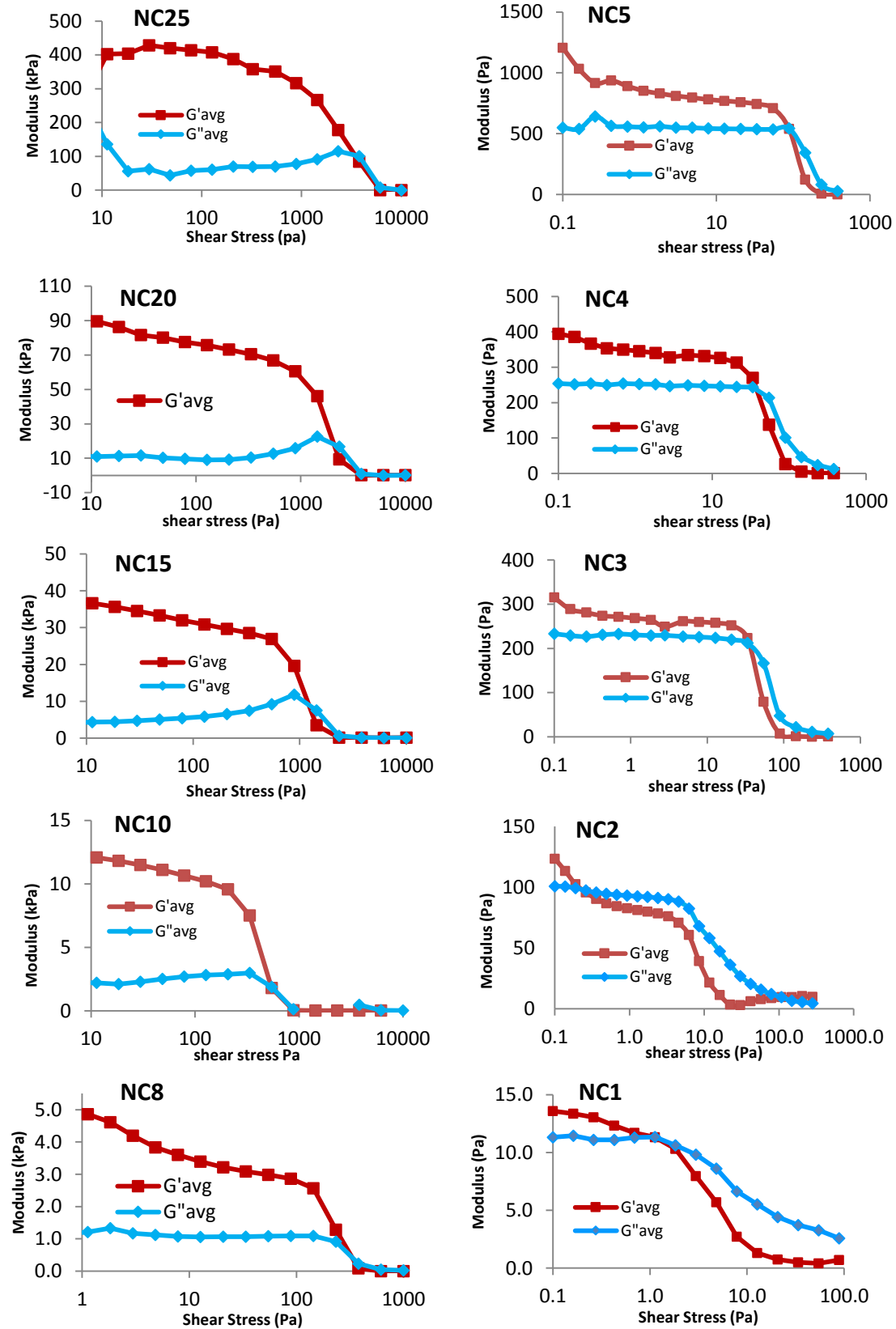
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Chapter 4.0

Rheology of Glycated Poly (*N*-isopropylacrylamide)-Clay Nanogels

4.1 Abstract

PNIPA-Clay nano composites prepared by free radical polymerization with clay are shown to be sensitive to glucose concentration by use of Oscillatory Shear Rheology(OSR) techniques. Samples were tested in the range of 0.0mg/ml to 3.0mg/mL which is within the range of physiological concentration**.** Increase in the shear relaxation modulus, G', was observed only in gels above the gel transition at physical glucose concentration of 1-2 mg/ml. Mesh size obtained through analysis of G_0 and relaxation exponent obtained from frequency sweep data indicates that the effect of physical property findings was due to a shrinking of the gel mesh size, which was restored when the glucose concentration was further increased to 3mg/ml. These results were interpreted in terms of a possible co-solvency between phases of different degrees of hydration. This information shows that effective tuning of PNIPA-Clay Nanogels requires mechanical response testing to provide better characterization of these gels for application such as drug delivery vehicles.

4.2. Introduction

PNIPA is a polymer hydrogel produced by free radical polymerization of NIPA using clay cross linkages[1]. The clay cross linkages are possible due to the initiator, KPS which has more affinity for the clay than the NIPA monomer allowing the NIPA to link to clay rather than link to itself. Gelation occurs as the number of clay platelets with attached monomer chains reaches the percolation threshold. We have previously shown that the concentration of clay required for percolated networks is approximately 15.24g/L for the gel described and used herein. PNIPA-Clay nanocomposites (NC) are strong, ductile and have a reversible phase change with a transition temperature around 34C[2]. Since this temperature is physiologically relevant, PNIPA has found multiple biomedical uses such as drug delivery systems[3, 4], rapid release cell culture substrates[1, 5], flocculation additives[6], separation devices[2], and wound healing dressings[7]. Since many of these applications place the material in direct contact with body fluids, it is important to also understand the behavior under physiological conditions. The behavior of PNIPA in the presence of different salts has been reported by numerous authors [8-11]. Salt changes the solvency of water shifting the phase transition temperature and water swelling of PNIPA. Glucose is another physiologically relevant substance, whose presence is in the blood and is correlated to diabetes[12]. According to the American National Diabetes Association, normal physiological concentration of blood glucose concentration ranges from 0.7mg/mL to 1.3mg/mL while the onset of diabetes occurs above 1.3mg/mL. In contrast to saline, very little research has been conducted on its effects on the properties of PNIPA gels. at these concentrations. Several authors have proposed using PNIPA as components of insulin

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drug carriers, which were shown to swell when glucose was introduced and where the degree of swelling was a function of glucose concentration, thereby releasing the insulin payload[13, 14], Kataoka et al. used a chemically cross linked poly-*N*-isopropylacrylamide derivatized with a definite fraction of a phenylboronic acid group as the glucose sensing moiety to show that gels were able to swell when exposed to glucose solutions spanning physiologically relevant levels. In both cases, it was proposed that the swelling action was due to secondary glucose binding components grafted i.e. phenylboronic acid grafted onto the PNIPA polymers or using these compounds as the cross linking element of the PNIPA gels **[15]**. In neither case was the influence of glucose on the unmodified PNIPA explored. Shpigelman[14] et. al. studied the effect of sugars on the collapse of PNIPA polymers proposed an elegant model for the decrease in the transition temperature of different stereo isomers based on their hydration number. In order to impact the LCST of these gels though, glucose concentrations larger than 10mg/ml which are beyond physiological concentrations were required. Here we report on the changes in the response of unfunctionalized PNIPA-clay gels when glucose is added at physiological concentrations which are below 4mg/mL. At these concentrations spectroscopic changes, as well as morphological changes in the polymer conformation are expected to be much smaller, and hence the effects were studied on a clay cross linked system, where these changes are amplified due to the collective effects of the cross linking junctions. Furthermore, rather than using spectroscopic methods we focused on the rheological response of the systems as a function of frequency and sheer stress. We found that even at these low concentrations modulation of the polymer structure occurred which could be explained in terms of a co-

solvency effect that occurs at low concentrations between water and partially hydrated glucose molecules.

Selection of the correct concentration of clay to produce the highest response from glucose is also important. As stated earlier we considered recent theoretical modelling of PNIPA nanocomposites which has shown that the percolation concentration of the clay filler, which coincides with the onset of gelation, occurs at approx. 15.24 g/l. We therefore chose to focus this study on PNIPA nanocomposites that bracket this value, with clay concentrations from 15.24g/L to 38.2 g/L, in order to determine the influence of glucose above or below the percolation transition.

4.3 Experimental

4.3.1 Materials.

N-isopropylacrylamide (95% purity) was from Sigma-Aldrich (St. Louis, Mo). Deionized water was obtained from a Barnstead E-Pure 3 stage water purification system with conductivity of 18.4 Meg-Ohms minimum. Residual oxygen was removed from the pure water by bubbling N_2 gas into water for 5 minutes prior to addition of ingredients. Synthetic hectorite; Laponite XLG was obtained from Rockwood Specialties Inc. (Chester, NY) with bulk density 1000 kg/m^3 and cation exchange capacity 104 mequiv/100g. Clay and NIPAs were grinded in mortar-pestle to obtain fine particles. Potassium Persulfate (KPS) and *N,N,N',N'*-tetramethylenediamine (TEMED), initiator and catalyst respectively, were obtained from Fisher Scientific (Fairlawn, NJ).

4.3.2 Synthesis of PNIPA-clay gels.

In making all samples we used 50 mL water, 5.65 g NIPAs (1moll/L), 50 mg KPA and 40 μL TEMED constantly. The clay concentration was 15.24g/L, 22.86g/L and 38.2g/L. Solids were mixed with water using a 5/8" turbine blade mixer at approximately 15,000 rpm. An ice-water bath was used to keep samples cool and below the lower critical solution temperature (LCST). This also allowed us to keep foaming to a minimum. Samples were refrigerated after mixing Clay and NIPA to cool them, then again after addition of KPS and lastly after addition of the TEMED with or without glucose.

4.3.3 Measurements

Rheological samples for each test point were generated by pipetting 2 mL of liquid into 37 mm petri dishes. Samples were then allowed to polymerize for a minimum of 24 hours in a covered glass bell jar. Rheological testing was performed using Bohlin HR-Nano Rheometer (E. Brunswick, NJ). Shear stress sweeps were performed using 2.0 cm plate geometry in contact with the gel surface inside the petri dishes. A normal (contact) force of 10.0 g was used to eliminate slipping. All samples were tested at constant temperature 25°C and constant frequency 1.0 Hz, while the shear stress ramps from low (0.1Pa) to high (1000.0Pa) until gel structure collapsed to very low values. Frequency sweeps were performed in a similar manner except that frequency was ramped from 0.1Hz to 100Hz at constant stress value of 10% of the measured yield onset (elastic region).

4.4 Results and Discussion

4.4.1 Oscillatory sheer rheology (OSR)

Data for two PNIPA clay compositions, 22.86 g/L and 38.20 g/L , which are above and below the gelation point, respectively, were performed as a function of glucose concentration for 0.0, 1.0, 2.0, 3.0 mg/ml are plotted in figures 1a-h (the data for higher glucose concentrations is provided in the supplementary materials). In figure 2a and 2b we plot the values of G', G", and the yield points obtained from these data as a function of glucose concentration. From the figure we can see that for low clay concentrations, below the gelation point, no influence of the glucose addition could be discerned in the rheological response. Above the gelation point, a significant increase, nearly 250%, in the dynamic modulus is observed with the addition of glucose, up to 2mg/ml. Further
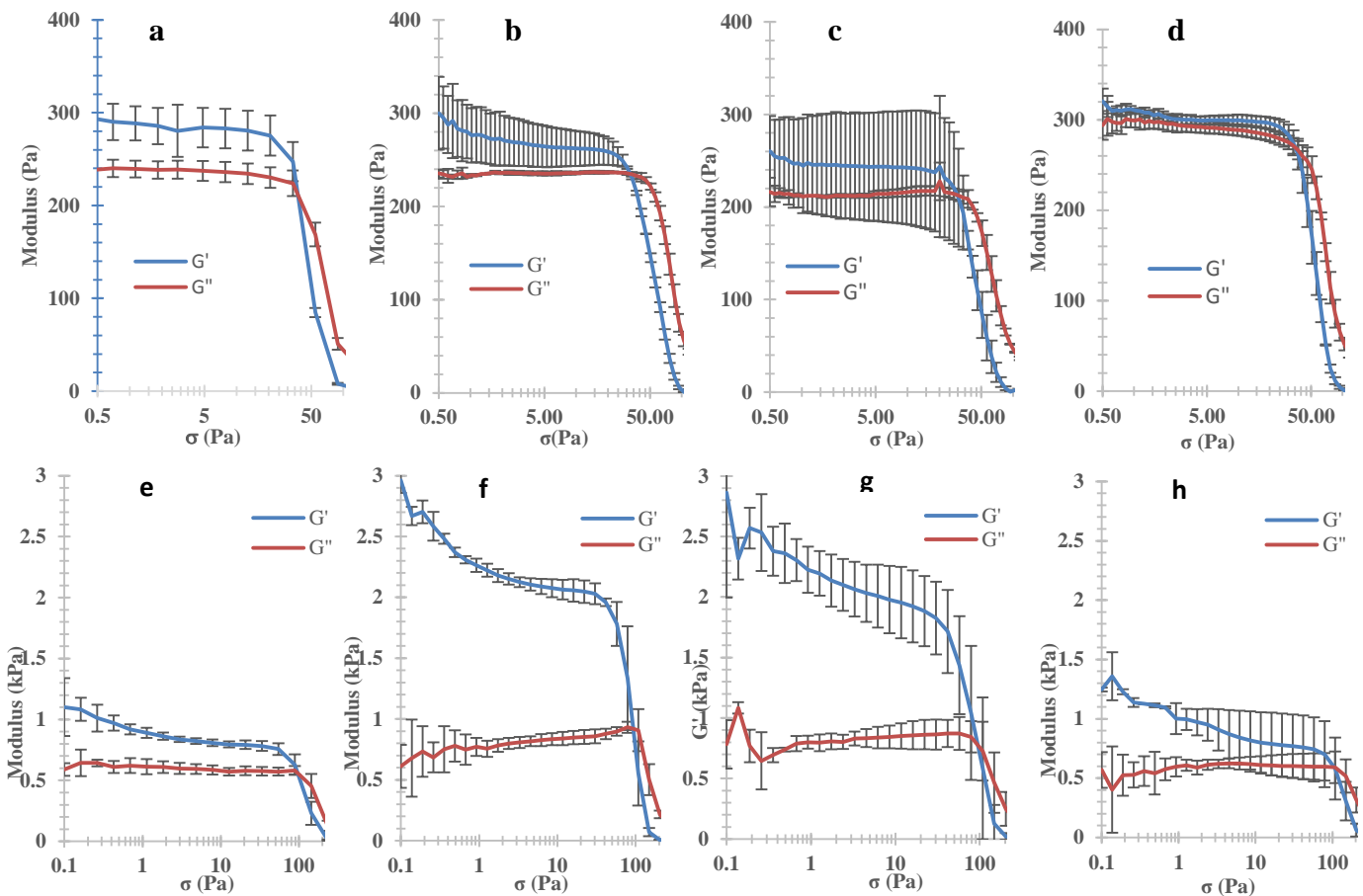
Figure 4.1a-h, Modulus vs. shear stress for PNIPA gels with 0.0.1.0, 2.0 and 3.0 mg/ml glucose ; a –d for clay concentrations of 22.86g/L , e-h for clay concentrations of 38.2 g/L .

addition of glucose, up 10 mg/ml restores the original value obtained in DI water

(supplemental data). No change with glucose is observed in either the viscosity or G" ,

and in the elasticity of the gel or the yield point, indicating that the observed effect is not
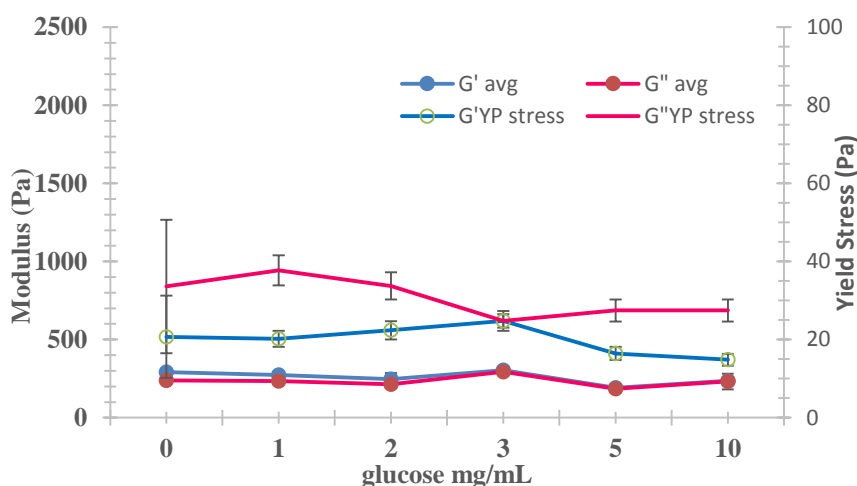
associated collapse of the gel.

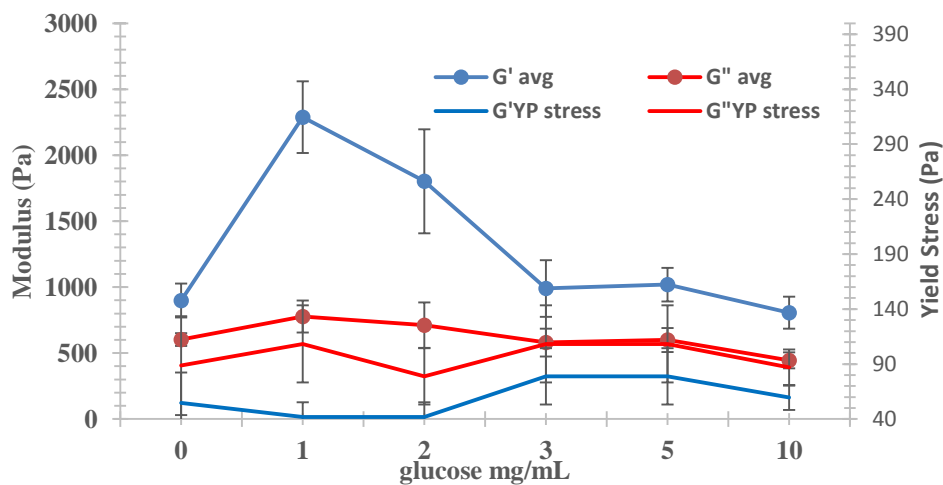**Figure 4.2a. Modulus and Yield Point vs. Glucose, 22.82 g/L clay**

Figure 4.2b. Modulus and Yield Point vs. Glucose, 38.2 g/L clay
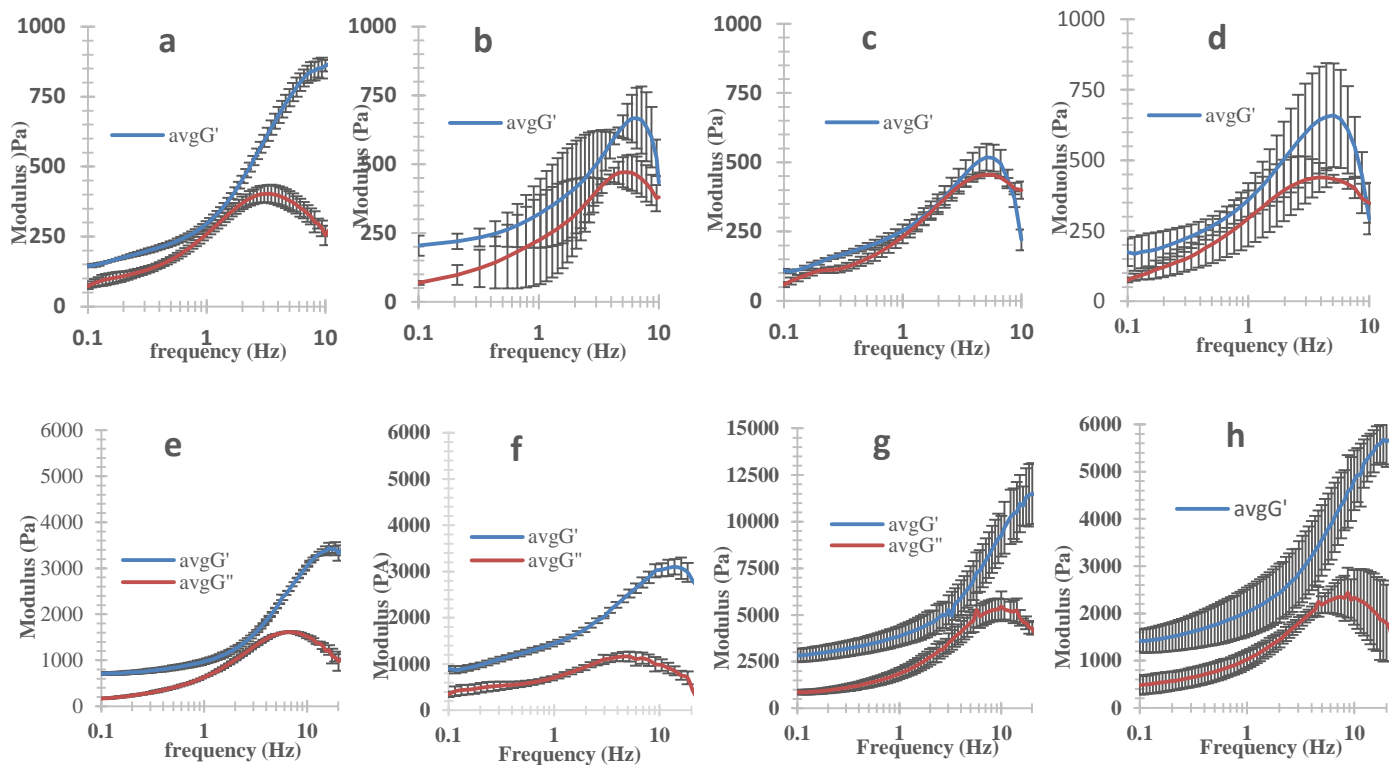
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Figure 4.3. Modulus vs. Frequency sweeps of 22.86g/L clay (a, b, c, d) and 38.2g/L clay (e ,f, g, h). Increasing glucose left to right 0mg/mL ,1mg/mL, 2mg/mL, 3mg/mL respectively

4.4.2 Frequency Sweep Analysis

In order to determine the correlation between the increase in modulus and the structure of

the network we also obtained frequency sweep data, plotted in figure 4.3.   From this data

we can obtain the low frequency modulus, Go, which is in turn related to the mesh size of

the network through,   Go= ($K_b T/\mathcal{E}^3$) Where Kb is Boltzmann's constant, T is temperature

and $\mathcal{E}$ is the correlation length between density fluctuations or the mesh size in a gel. The

values obtained for the two PNIPA gels as a function of glucose concentrations are plotted
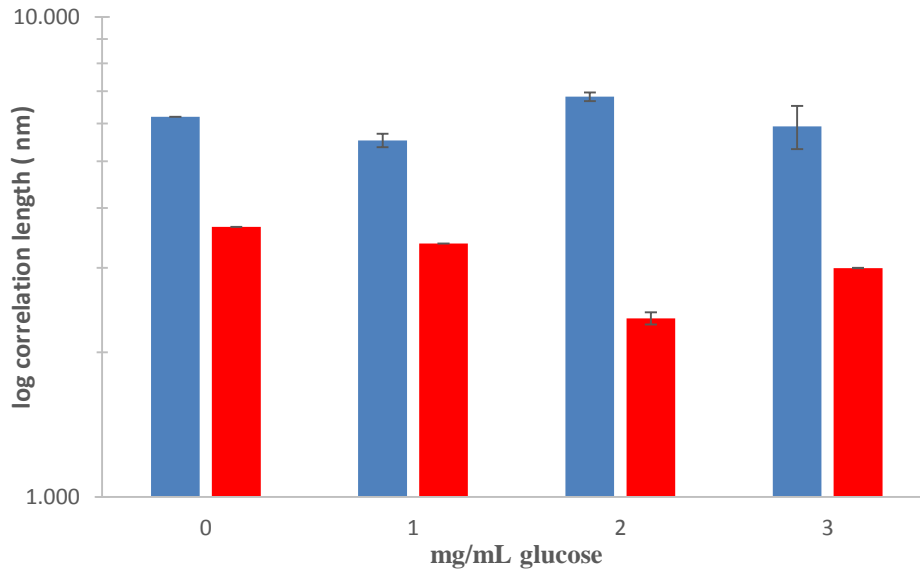
in figure 4.4.

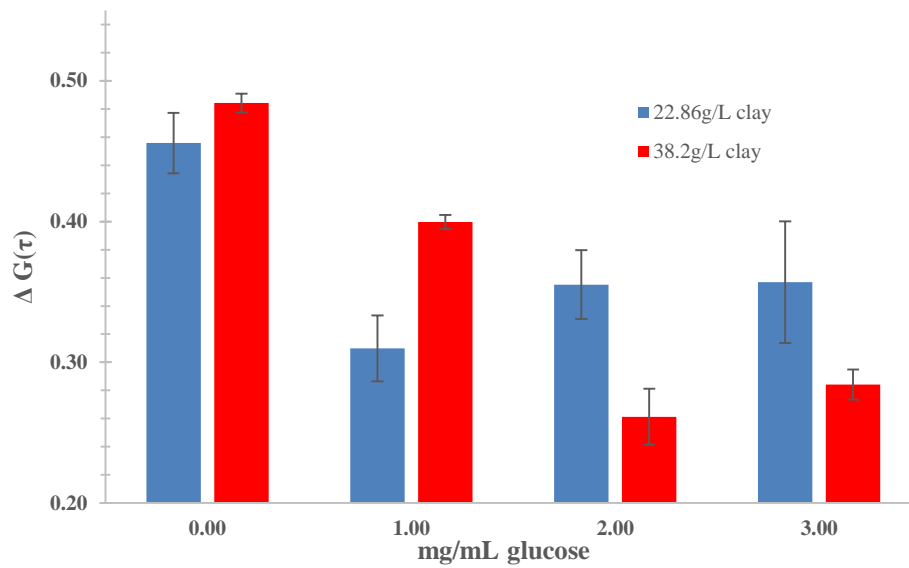**Figure 4.4a Change in correlation length (ε) with glucose**

Figure 4.4b Change in Δ power law exponent with glucose

From figure 4.4a we find that, as expected, at these low glucose concentrations, the mesh size or the structure of the polymer is unaffected for the low clay concentration sample. On the other hand, from the figure we can see that the mesh size decreases by more than 25% with the addition of 2mg/ml of glucose. Addition of glucose at a concentration of 3mg /ml restores the original mesh size. The time/frequency response of the relaxation modulus

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is also related to fluctuation times of the system, $G(t) \sim t^{\Delta}$[16] where t is proportional to the angular frequency measurement. The relaxation time of the system is plotted in figure 4.4b where we can see that in this case the presence of glucose, rather than the mesh size results in a reduced value.

4.5 Explanation of glucose effect

These results indicate that the change in mesh size is not related to coil/globule transition, which was reported to occur in PNIPA at much high concentrations[14]. A possible explanation for the observed phenomena is illustrated in figure 5 based on the theory of [17-19], which have proposed that when two solvents are present, each an ideal solvent for a polymer, but good solvents for each other, the polymer molecules become excluded from the solvent volume when the two are together in stoichiometric volumes. The effect occurs since the polymer chains maintain their Gaussian conformation when dissolved in either two solvents. But when the solvents are present together, their attraction for each other far exceeds the attraction for the polymer, there excluding the polymer and reducing the polymer radius. The region where this occurs corresponds to the concentration which exactly matches the "sphere of inclusion" of the solvent molecules or in this case the hydration shell . When the solvents are mixed they will form the hydrated complex and solvate the polymer only when the concentrations are such that excess volume remains of either of the two solvents. This principal was illustrated using water and alcohol solutions [17] .

It has been shown by several groups that glucose and water forms a hydration complexes where the chemical potential is a function of the degree of hydration. We can then postulate that a concentration can be established where the ideal degree of hydration is

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reached. For our data this value would be in the vicinity of 2mg/ml or in the physiological range. For an uncross linked polymer solution this would results in a small change in polymer radius of gyration, which would be difficult to detect. In a cross linked systems on the other hand, even a small change has a collective effect on the mesh size of the systems, which can then be detected via measurement of the dynamic modulus. Since this effect occurs within the physiological range of glucose it may have significant consequences in such gels as those made by fibrinogen, hyaluronic acid or collagen, where they impact the prognosis of patients with diabetes in multiple pathologies.

4.6 Conclusion

We have performed oscillatory shear measurements on PNIPA/Clay nanocomposites well above and near the gel transition as a function of glucose in the concentration range of 0-10mg/ml.

A large increase in the shear relaxation modulus, G', was observed only in gels well above the gel transition at physical glucose concentration of 1-2 mg/ml. Extrapolating to obtain the low frequency modulus Go, we found that the effect was due to a shrinking of the gel mesh size, which was restored when the glucose concentration was further increased to 3mg/ml. These results were interpreted in terms of a possible co-solvency between phases of different degrees of hydration.

4.7 Supplemental Information

Figure 5. *G'* and *G"* results for PNIPA-clay gels at glucose concentrations of 5.0 and 10.0 mg/mL for clay concentrations of 22.86g/L and 38.2g/L.
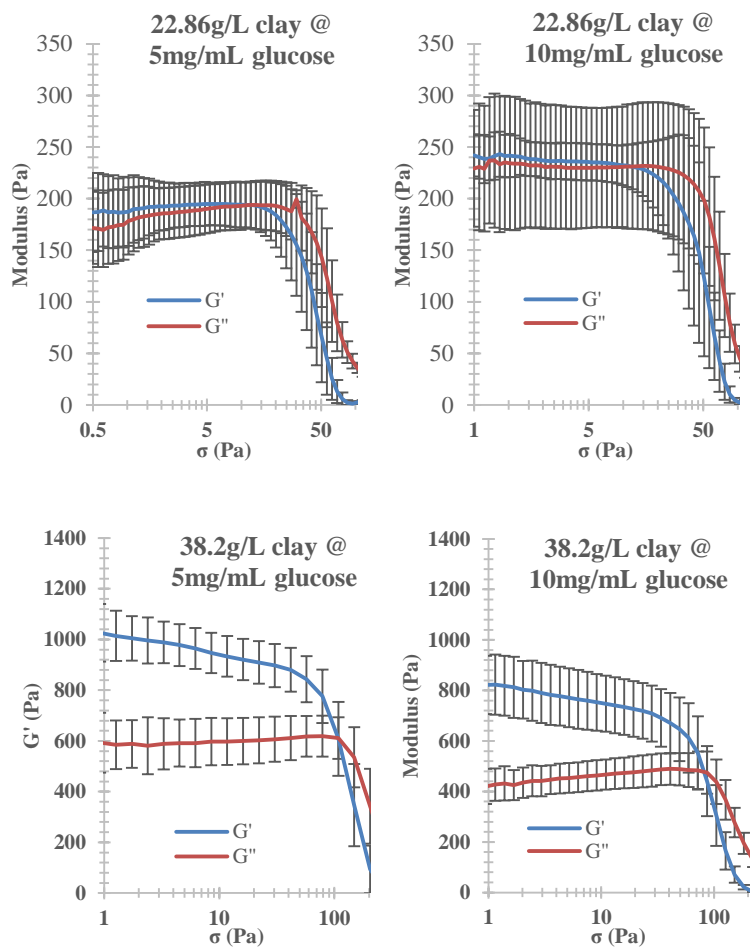

Figure 5. Modulus vs. shear stress at 22.86g/L clay and 38.2g/L clay with 5mg/mL and 10mg/mL glucose

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