

Entanglement of thread-like structures embedded in liquids and gels and its effect on linear and nonlinear viscoelasticity.

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Abstract

This PhD project studied the relationship between mesoscopic structure and linear and nonlinear rheological behavior of thread-like systems, mainly living polymers and biopolymer gels. The study was realized from an experimental physics point of view using rheometric and multiple light scattering as principal experimental techniques. This work is divided into 5 Chapters: Chapter 1, "Introduction," Chapter 2, "Theory," Chapter 3, "Experimental Techniques," Chapter 4 as a principal section, "Results", and Chapter 5, "General Conclusion and Perspectives."

Living polymers and biopolymer gels suffer interesting macroscopic phase transitions by modification of the environment. These systems have been studied in different stages where they can relax as liquid-like and solid-like, depending on protocol measurement or physicochemical environments. The principal chapter shows the experimental results and their discussion divided into three subsections, taking wormlike micelles solutions as a model of living polymer, gelatin solutions as flexible polymer, and chitosan and alginate as charged flexible polymers. The linear and nonlinear rheological measurements were performed through SAOS and LAOS protocols. Diffusive wave spectroscopy micro-rheology was also performed to measure the mean square displacements of microspheres embedded in thread-like fluids to obtain information about the relaxation mechanism of chains and establish the connection with the viscoelasticity properties. For these thread-like systems, this research has revealed a crucial insight that the structure of the polymer matrix directly influences the nonlinear rheological response. The Lissajous-Botwich curves serve as a fingerprint for each system. For living polymers, a significant discovery was the definition of the entanglement index, an essential mesoscopic parameter used for understanding the entanglement of the micellar network. This index is directly linked to the solution's elasticity from a dynamic fluid point of view. The study of large shear strain rheological behavior in biopolymer gels indirectly reveals the fractal dimension, which agrees with the dimension obtained directly from other experimental techniques. An important finding is that the Chambon & Winter criteria show a similar critical exponent ($n \sim 0.6$) for all physical gels, indicating that the percolation threshold of the network is independent of gelation mechanisms. Finally, the mixture of alginate and chitosan forms microgels, which exhibit the same rheological signature as coreshell synthetic microgels, possibly due to the gel preparation method.

Resumen

Este proyecto doctoral estudia la relación entre la estructura mesoscópica y el comportamiento reológico lineal y no lineal de sistemas filamentosos, principalmente polímeros vivientes y geles biopoliméricos. El estudio se realizó con un enfoque experimental utilizando la reometría y la dispersión múltiple de luz como principales técnicas. La tesis doctoral se divide en 5 capítulos: Capítulo 1, "*Introducción*", Capítulo 2, "*Teoría*", Capítulo 3, "*Técnicas experimentales*", Capítulo 4 (sección principal), "*Resultados*", y Capítulo 5, "*Conclusiones generales y perspectivas*".

Los polímeros vivos y los geles de biopolímeros sufren interesantes transiciones de fase por modificación del entorno. Estos sistemas se han estudiado en diferentes etapas donde pueden relajar como líquidos o sólidos, dependiendo del protocolo de medición o el entorno fisicoquímico. El capítulo principal muestra los resultados experimentales y su discusión dividida en tres subsecciones, tomando soluciones de micelas en forma de gusano como modelo de polímero vivo, soluciones de gelatina como polímero flexible, y quitosano y alginato como polímeros flexibles cargados. Las mediciones reológicas se realizaron mediante SAOS y LAOS. La microrreología se realizó usando espectroscopia de onda difusiva para medir los desplazamientos cuadráticos medios de microesferas incrustadas en la matriz filamentosa, y así obtener información sobre el mecanismo de relajación de las cadenas y establecer una conexión con la viscoelasticidad. Para estos sistemas, esta investigación muestra que la estructura de la matriz filamentosa influye directamente en la respuesta reológica no lineal. Las curvas de Lissajous-Botwich sirven como huella digital para cada sistema. En el caso de los polímeros vivientes, la definición del índice de entrelazamiento, un parámetro mesoscópico, es esencial para comprender el enredamiento de la red. Este índice está directamente relacionado con la elasticidad de la solución desde el punto de vista de la dinámica de fluidos. El comportamiento reológico no lineal de los geles revela indirectamente la dimensión fractal, que concuerda con la dimensión obtenida por otras técnicas experimentales. Un hallazgo importante es que el criterio de Chambon y Winter muestra un exponente crítico similar (n \sim 0,6) para todos los geles físicos, lo que indica que el umbral de percolación de la red es independiente de los mecanismos de gelificación. Finalmente, la mezcla de alginato y quitosano forma microgeles, los cuales presentan la misma firma reológica que los microgeles sintéticos de núcleo-capa, posiblemente debido al método de preparación del gel.

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1. Introduction

The term *soft matter* was popularized in 1991 by Nobel laureate Pierre Gilles de Gennes to refer to materials that exhibit large deformations in response to moderate forces. In soft matter systems, the characteristic activation energy per particle is of the order of room-temperature energy ($\sim k_BT$; k_B is the Boltzmann constant, and T is the absolute temperature with $T \sim 300 K$), and under this definition, the range of materials is almost limitless. A long list begins with polymers, liquid crystals, colloids, emulsions, foams, pastes, and fluids formed by water mixtures with surfactants or amphiphiles. Still, it also includes ferrofluids, granular materials, biological materials, active fluids, and many more [1]. One way to visualize the soft matter materials is by the "*soft matter triangle*" (see Fig. 1.1), which shows a continuum of molecules and materials that fills the space between spherical colloids, flexible polymers, and amphiphiles [2].



Figure 1.1 Soft matter triangle. Image from [2].

On the right-hand side of the soft matter triangle, from amphiphiles to flexible polymers, it is possible to see a large variety of materials that share a common characteristic: elongation. Due to electrostatic forces (van der Waals forces) and entropic interaction, the self-assembly of several amphiphile molecules can form macromolecular complex structures at a mesoscopic scale (from hundreds of nanometers to a few micrometers). On the other hand, polymers are chains formed by many monomers linked through covalent crosslinks; in both cases, it is possible to see the mesoscopic structure as a thread-like structure like spaghetti. These complex structures result in dynamics usually described in multiple characteristic lengths and time scales and are described as complex fluids due to the complexity of its flow. If the microscopic structure of a complex fluid is perturbed from its equilibrium state, its behavior can be viscoelastic; the mechanical response involves a solid behavior due to the recovery of the mesoscopic elements to their initial condition and a liquid behavior when the mesoscopic elements flow after recovery [3].

Usually, the viscoelastic properties of complex fluids can be determined by measuring the response due to one stimulus. Inherently, the viscoelasticity response rises from mesoscopic structure, which is the soft matter paradigm. The connection between mesoscopic structure and viscoelasticity is known in some detail when the response is linear, e.g., using the Small Amplitude Oscillatory Shear protocol (SAOS), where the shear stress is measured due to the small oscillatory shear strain applied. However, the relationship between mesoscopic structure and nonlinear viscoelasticity is unclear. This represent a recent soft matter problem due to recent technological developments, e.g., a refinement in the rheometric measurements; the best example is to perform the Large amplitude Oscillatory Shear (LAOS) protocol [4]. The possibility of modifying the viscoelastic properties by controlling the physicochemical environment of thread-like materials has been of special interest in the soft matter community. The correlation between the threadlike structures in the linear and nonlinear viscoelasticity of these systems is far from being fully understood, and their compression is of particular interest to the Complex Fluids Group at the Institute of Physics at UNAM. The principal aim of this work is the study of the effect of thread-like structures, taking living polymers and biopolymer gels as models in their linear and nonlinear viscoelasticity. Below is a brief introduction to living polymers and polymer gels.

1.1 Living polymers

Several theories have been proposed to connect the macroscopic and rheological behavior of complex fluids with the microscopic properties of entangled polymers. Among the most successful ones are the reptation, tube theories, and their extensions [5]. One of the strongest assumptions of these theories is that polymer chains do not change their architecture, length, or topology within experimental timescales; if the systems do not obey the last constraint, they are called *"living polymers."* Living polymers are different from their non-living counterparts as random architectural changes such as breakage, fusion, and reconnections alter the architecture and topology of the polymers on timescales similar to their relaxation times [6].

Wormlike micelles (WLMs) are examples of living polymers. These systems are elongated, flexible cylindrical aggregates in aqueous solutions formed by the self-organization of amphiphiles. WLMs have contour lengths of a few micrometers $(1 - 20 \ \mu m)$ and a cross-section of a few nanometers $(1 - 12 \ nm)$, where the persistent length is lower than the contour length [7]. Above a threshold concentration, their entanglement into a transient network provide remarkable viscoelastic properties reminiscent of polymer solutions or gels, distinguishing two new lengths: entanglement length and mesh size. According to the reptation–reaction model, an average chain length dominates relaxation if the micellar breaking and reformation processes are faster than their reptation; if a shear strain is applied, the shear stress relaxes as in the Maxwell model, G(t) = $G_0 exp(-t/\tau_R)$ at long times, or equivalent, low and intermediate frequencies (ω) in its related complex modulus $G^*(\omega) = i\omega \mathscr{F}[G(t)] = G'(\omega) + iG''(\omega)$. The elastic modulus, G_0 , and the relaxation time, τ_R , can be obtained from the linear viscoelastic spectrum from the crossing between the $G'(\omega)$ (elastic modulus) and $G''(\omega)$ (viscous modulus) curves [8].

When WLMs solution is subject to a stationary flow, band formation may occur, *i.e.*, the transition from a homogeneous to an inhomogeneous flow state. Macroscopically, bands with different shear rates coexist in the fluid, and their formation occurs in the direction of the velocity gradient. Shear banding is similar to a nonequilibrium phase coexistence between an isotropic and strongly aligned phase (paranematic), which has a lower viscosity than the quiescent phase [9,10]. In a simple shear banding scenario, the fluid is divided into two macroscopic regions separated by a thin, steady interface of finite width. Many WLMs solutions present this simple scenario phenomena [9,11]; however, other WLMs present inhomogeneous flow and cannot be classified as a simple scenario of shear banding, e.g., CTAB/NaSal system (R = [NaSal]/([CTAB] = 2), [CTAB] = 100 mM) present multiple shear banding [12].

The viscoelastic spectrum given by SAOS has been extensively studied to quantify micellar systems' viscoelasticity properties. However, interesting nonlinear effects occur if the strain amplitude is increased, e.g., using the LAOS protocol [4]. An appealing feature of LAOS in Maxwellian fluids is that the hardness of the flow's time dependence, relative to the fluid's intrinsic

relaxation timescale, can be tuned by varying ω in the applied oscillation without sudden jumps in the strain input, as in step experiments [13]. LAOS experiments can explore the full range between steady-state and strongly time-dependent behaviors by investigating the effect of two characteristic dynamic variables, the Deborah number, *De*, and the Weissenberg number, *Wi* [4] In addition, in LAOS, shear banding has been experimentally observed [14]. Some models have been capable of predicting shear banding in the LAOS protocol, such as those using the partialextending convected equation [15] or the VCM model (Vasquez-Cook-McKinley) [16]. However, to study shear banding theoretically in different experimental protocols, the model must include nonhomogeneous flow, and a criterion for the onset constitutive curve of stress as a function of strain rate is nonmonotonic [17].

In simple fluids (called Newtonian fluids) in steady shear flow, the flow tends to become unstable and eventually turbulent because of inertia when the Reynolds number, $Re = \tau_i \dot{\gamma}$, significantly increases; $\dot{\gamma}$ is a characteristic deformation rate; $\tau_i = \rho d^2 / \eta$ is the viscous diffusion time, where d is a characteristic length in the velocity gradient direction, η is the viscosity, and ρ is the mass density [18]. In non-Newtonian fluids, as in WLMs solutions, the stress relaxation brings up an additional timescale τ_R . Here, another route to instabilities exists, which does not involve inertia and is referred to as purely elastic. If $Re \ll 1$, the Wi number takes the role of the control parameter that can lead to the emergence of secondary flows and eventually to elastic turbulence. If both Re and Wi are large, flow instabilities are referred to as inertio-elastic. One form to characterize the inertio-elastic instabilities is by measuring the Taylor number should be written as $Ta = \Lambda^{1/2} f(Re, Wi)$, with Λ is the curvature of the streamlines. When f goes to Re when $El \to 0$, and f goes to Wi when $El \rightarrow \infty$; here, $El = Wi/Re = \tau_R/\tau_i$ is the elasticity number, which is the ratio of elastic forces to inertial forces within a fluid [16,19]. Re and Wi numbers are rates between different forces involved in the flow dynamics of a fluid. However, from a different perspective, they do not measure any intrinsic property related to the micelle network, which is responsible for the elastic forces in the fluid when it is deformed during the flow.

In the semi-dilute region, micro-rheology using Diffusion Wave Spectroscopy (DWS) has provided estimations for the relevant scales of the micellar network [20–22]. The general principle behind micro-rheology is to minimize the mechanical probe that deforms the medium, such as a

colloidal microsphere. So, the material's properties can be extracted by tracing the motion of the thermally fluctuating probes in some way. Diffusing Wave Spectroscopy (DWS) microrheology allows the measurement of the mean square displacement of the tracers; the rheological material properties at high frequencies can be obtained from it, through the micro-rheology equations [23]. If the flow process is very slow, one could expect that the micellar network slowly disentangles, contributing to some extent to the elastic forces involved in the flow. However, if the micellar network is deformed fast, not allowing enough time for the tubular micelles to disentangle due to the speed of the imposed strain, the network's contribution to the elastic forces involved in the flow would increase. One quantity that somehow measures the relative entanglement of a micellar network would be the ratio of the total contour length of the micelles in the network to their entanglement length, which can now be experimentally estimated.

1.2 Polymer gels

The flexible polymers share a similar structure to WLMs. Above a threshold concentration, it is possible to see the same structural lengths as WLMs; however, relaxation dynamics are more complex, with several relaxation times; e.g., the viscoelasticity behavior might be described as a sum of several Maxwell forms' contributions [5,24]. These complexities in the structure and dynamics confer the possibility of seeing several phenomena as inertio-elastic instabilities [25] and classical effects such as Weissenber, Barus, and Fano phenomena [24]. Beyond the viscoelasticity properties of polymer solutions, one interesting property of polymers is the capacity to form gels (gel state); gels are disordered solids from forming a 3D infinity network by physical or chemical bonding of the constituent chains of the precursor solution (sol state).

A gel is an infinite polymeric network formed by molecules linked together, creating tridimensional branched structures similar to disordered lattices. According to the nature of the linking molecules, gels are commonly classified as chemical or physical gels. In physical gels, bonds are reversible when thermodynamic parameters such as pH, ionic strength, or temperature are modified. Physical junctions are constantly created and destroyed at very low rates, and the network seems to be permanently connected if the observation time is shorter than the bond's lifetime [26]. On the other hand, chemical reactions form chemical gels, where permanent covalent bonds are created, and as a consequence, gelation is irreversible. Furthermore, it is

feasible to form hybrid gelatin gels by mixing crosslinking processes, *i.e.*, physical or chemical, producing the so-called physicochemical gels, which present some partial reversibility; they are not entirely thermoreversible.

The gel transition can be described using the percolation model, where monomers occupy all possible sites in a lattice with functionality equal to the number of closest neighbors. Bonds are formed randomly with a probability, p. When $p < p_{c_i}$ only small clusters are formed (sol state); conversely, if $p > p_{c_r}$ an infinite cluster appears (gel state). Depending on the connectivity mechanism, the gel point hallmarks the transition from a liquid to a solid due to gelation; a polymer at its gel point is commonly called a critical gel to distinguish it from the various materials named gels [26]. A critical gel is far beyond from equilibrium due to the diverging internal length scales, which cause very slow structural rearrangements. A large number of rheological experimental studies for a large variety of physical or chemical gelling materials in the linear regimen indicate that the complex shear modulus follows a power law in the angular frequency [27,28], *i.e.*, $G'(\omega)$ = $G_c'\omega^n$ and $G''(\omega) = G''_c\omega^n$; n is a critical exponent, G_c' and G''_c are constants, and the loss angle $\delta = tan^{-1}[G''(\omega)/G'(\omega)] = n\pi/2$. These formulas are helpful for the gel point detection because $G'(\omega)$ and $G''(\omega)$ must be parallel to each other, which consequence is the frequency independent of $tan(\delta)$ to be frequency independent. At the critical gel, the rich rheological diversity of a material converges to a universal dynamical state of less rheological complexity, exhibiting a simple selfsimilar relaxation behavior, $G(t) = \mathscr{F}{G(t)/i\omega} = St^{-n}$, where G(t) is the relaxation modulus, and S and n are two fitting material parameters characterizing the gel [27,28]. The nature of the links and their quantity impact the network structure of a gel, and consequently, its deformation and elasticity properties could change dramatically from linear to nonlinear regimes; the differences and similarities are not fully understood in the nonlinear viscoelasticity regime. Similar to the WLMs solution, the persistence length of the unstructured, non-bonded flexible polymer sections and the network's mesh size decreases due to linking formation and could be measured using the DWS-microrheology technique.

In nature, there is a wide variety of polymers that may form a gel; if monomers are amino acids, the polymer is called a protein; if the monomers are sugars, the polymer is called a polysaccharide; in both cases, the term polymer change to biopolymer. Inherently, the self-

assembly of biopolymers depends on the nature of the monomers and the interaction between them, raising a large variety of complex structures embedded in the medium. Gelatin is an excellent system for studying physical or chemical gelation and the competition among physical and chemical mechanisms to form hybrid gels with flexible polymers. One advantage is the wellknown detailed self-assembly transition from single-strand to triple-helix chains and chemical gelation crosslinking mechanisms with glutaraldehyde [29-31]. Depending on the sample preparation, temperature lowering, adding glutaraldehyde, or doing both things simultaneously, physical, chemical, or hybrid gels can be obtained. This flexibility to form different gels from a single molecule makes gelatin ideal for studying critical and post-gel states in flexible biopolymers with different crosslinking mechanisms. Comprehension of the competition between physical and chemical mechanisms could probably be exploited to understand the evolution of mechanical properties and mesoscopic scales of chemical gelatins formed with different chemical crosslinkers such as genipin [32], glyceraldehyde [33], bisvinylsulfonomethyl [34], as well as those using an enzymatic route [35,36], or in biopolymers undergoing natural gelation processes, where secondary structures are formed from single polymer molecules in a sol state, such as polysaccharides, alginate, pectin, and carrageenans [37].

If the polymers are polyelectrolytes, i.e., their monomers are negatively or positively charged, electrostatic interactions between the chains can trigger the appearance of soluble and insoluble complexes and the formation of coacervates (a dense liquid phase); these structures are called PECs (polyelectrolyte complex). The formation of PECs gives rise to anisotropies, and their formation can be controlled by the variation of physicochemical parameters [38]; however, it is possible to form uniform gels under certain thermodynamic conditions. The formation of gels and membranes with mixtures of polyelectrolytes has been reported, e.g., chitosan/alginate mixture [39–42]. These two natural polyelectrolytes are of great interest due to their natural availability and biocompatibility for many biomedical applications [38]. From a fundamental question, it is unknown if the physical and chemical gels share the same critical exponent [27]; here, the polyelectrolytes form a gel by physical mechanism differently from the physical gelatin gel. So thus, a the question arises on the possibility of keeping the critical exponent is the same for all types of gels independently of crosslinking mechanisms. Additionally, no in situ work has been reported on

the formation of PECs during gelation and their impact on viscoelastic properties has been studied. DWS-microrheology is a good experimental technique to study the structural evolution of the network, and describes how are the different relaxation modes at high-frequency regimens.

1.3 General and specific goals

In general term

The study of the effect of thread-like structures in the linear and nonlinear viscoelasticity of solutions and gels in different physicochemical conditions using rheometric and multiple light scattering as principal experimental techniques.

In specific terms

- To obtain the linear and nonlinear viscoelasticity behavior of Wormlike Micelles (WLMs) solution made with different amphiphiles and to correlate the mechanical response with the elasticity forces. Using Diffusive Wave Spectroscopy (DWS) micro-rheology, to measure the mean square displacement of microspheres embedded in the micellar fluids to obtain the high-frequency viscoelasticity spectrum and, subsequently, the mesoscopic characteristic lengths of the WLMs. Using the information on characteristic lengths, rheological response, and elasticity, to find the relationship between elasticity and a mesoscopic length parameter to understand the origin of the contribution of elastic forces from a molecular point of view in the dynamics of WLMs solutions.
- To obtain the linear and nonlinear viscoelasticity behavior of gelatin gels like flexible polymer model in different situations, depending on the process of forming their links: physical, chemical, or hybrid. The viscoelasticity properties correspond to the gel point and mature estate. DWS microrheology technique is used for kinetic description regarding the relaxation of chains and their mesoscopic lengths at different times before starting the gelation process. With these characterizations, it is possible to obtain the connection between the mesoscopic evolution of the network with the viscoelasticity properties and some network parameters, such as a critical exponent (*n*) in the gel point and the fractal dimension (*d_f*) in the mature gel.
- ✤ In the same way as gelatin gels, obtain the linear and nonlinear viscoelasticity behavior of chitosan, alginate, and their mixture are obtained using a flexible charged polymer model in different situations; the linking process is physical. Using DWS microrheology results, to establish a connection between the mesoscopic evolution of the network with the viscoelasticity properties and some network parameters such as *n* and *d_f* parameters.

2. Theory

2.1 Theory of polymers

The term polymer defines a molecules formed by many elementary units, called monomers connected by covalent bonds. Generally, these macromolecules are made up of between 20 to 10 million monomers. Polymers formed by less than ten monomers are called oligomers. In addition, if the polymeric chain is made up of monomers of the same type, it is called a homopolymer. On the contrary, if the monomers are of different types, they are called heteropolymers. Other side, many shapes may that polymeric chains can adopt. In particular, there are two simple cases: 1) Flexible polymers where, for a sufficiently large scale, the polymer adopts a shape like spaghetti, and ii) as a rigid rod. Both cases are shown in Fig. 2.1. [5]. The above cases are extreme, and intermediate cases, e.g., semi-flexible polymers, can be found.



Figure 2.1. a) Flexible polymer, b) rigid rod polymer, c) and d) their concentrated solutions, respectively. Image from [5].

Polymer solutions are obtained by dissolving a polymer in a solvent. They are classified based on the volume fraction $\phi = c/\rho$, where *c* represents the polymer mass concentration and ρ denotes the density. If ϕ is less than the overlap volume fraction ϕ^* (the concentration at which there is no crosslinking of the chains), the polymer solution is categorized as dilute. Conversely, when $\phi > \phi^*$, the solution is considered semi-dilute, indicating the presence of overlap [43].

2.1.1 Flexible polymers

A polymer chain can be characterized by a specific stiffness, and qualitatively can be described by the ratio of two characteristic lengths: The contour length, L_{c} , and the persistence length, I_{p} . The contour length is the length of the entire polymeric chain, and the persistence

length is understood as the rigid section of the chain. It is related to the elastic modulus of curvature κ as $l_p \approx \kappa (k_B T)$. Considering the extreme cases, when $L_c \gg l_p$, we are dealing with flexible polymers, and, on the contrary, if $L_c \ll l_p$, we speak of rigid bar-type polymers. The persistence and contour lengths are similar in semiflexible polymers, *i.e.*, $L_c \approx l_p$.

For flexible polymers, the relationship between the scale's lengths is such that the polymer is simulated as a random walker with a step length, $b (\sim l_p)$ smaller than the polymer's total length. Under these conditions, at room temperature, it is possible to study the polymer with a Langevin approximation in which the polymer constituents are constantly subjected to random forces given the beating of the solvent molecules with the polymer chain.

The Rouse model. The dynamic of polymer solutions can be described by a set of beads connected along the chain, under the assumption that their dynamic is Brownian. This model was proposed by Rouse [69] and has been the basis for the dynamics of dilute polymeric solutions. The following discussion can be seen in detail in the textbook *The Theory of Polymer Dynamics* by M. Doi & S. F. Edwards [5]. If $\{R_n\} \equiv (R_1, R_2, ..., R_N)$ are the positions of the beads, the equation of motion describing the polymeric chain is:

$$\frac{\partial}{\partial t}\boldsymbol{R}_{n}(t) = \sum_{m} \boldsymbol{H}_{nm} \cdot \left(-\frac{\partial U}{\partial \boldsymbol{R}_{m}} + \boldsymbol{f}_{m}(t)\right) + \frac{1}{2}k_{B}T\sum_{m}\frac{\partial}{\partial \boldsymbol{R}_{m}} \cdot \boldsymbol{H}_{nm},$$
(2.1)

where $f_m(t)$ is the stochastic force, and H_{nm} is the mobility tensor. The last relationship is referred to Langevin equation. It is important to note that it is not considered the inertia of the polymer chain, so the second derivative concerning time disappears.

The Rouse model considers a harmonic potential and does not take into account the excluded volume interaction (interaction between two segments of the same chain) and hydrodynamic interactions considering that the chain is surrounded by vacuum. The mobility tensor and the interaction potential can be written as:

$$\boldsymbol{H}_{mn} = \frac{\boldsymbol{I}}{\zeta} \delta_{nm}, \tag{2.2}$$

$$U = \frac{k}{2} \sum_{n=2}^{N} (\mathbf{R}_n - \mathbf{R}_{n-1})^2 \text{ with } k = \frac{3k_B T}{b^2}$$
(2.3)

where *b* is the link length between two beads, and ζ the mobility. In this model, the Eq. 2.1 becomes a linear equation for R_n . For internal beads (n = 2, 3, ..., N-1),

$$\zeta \frac{d\boldsymbol{R}_n}{dt} = -k(2\boldsymbol{R}_n - \boldsymbol{R}_{n+1} - \boldsymbol{R}_{n-1}) + \boldsymbol{f}_n.$$
(2.4)

And, for end beads (n = 1, and N):

$$\zeta \frac{d\mathbf{R}_1}{dt} = -k(\mathbf{R}_1 - \mathbf{R}_2) + \mathbf{f}_1 \quad \& \quad \zeta \frac{d\mathbf{R}_N}{dt} = -k(2\mathbf{R}_n - \mathbf{R}_{n+1} - \mathbf{R}_{n-1}) + \mathbf{f}_n.$$
(2.5)

The distribution of $f_n(t)$ is Gaussian, characterized by the first and second moment as:

$$\langle f_n(t) \rangle = 0 \quad \& \quad \langle f_{n\alpha}(t) f'_{m\beta}(t') \rangle = 2\zeta k_B T \delta_{nm} \delta_{\alpha\beta} \delta(t - t'). \tag{2.6}$$

The suffix *n* in the Rouse model can be regarded as a continuous variable. In the continuous limit, the Eq. 2.4 is written as:

$$\zeta \frac{\partial \boldsymbol{R}_n}{\partial t} = k \frac{\partial^2 \boldsymbol{R}_n}{\partial n^2} + \boldsymbol{f}_n.$$
(2.7)

The Eq. 2.5 is included in the last equation if the hypothetical beads R_0 and R_{N+1} are defined as R_0 = R_1 and $R_{N+1} = R_N$, respectively, which become in the continuous limit as:

$$\frac{\partial \boldsymbol{R}_n}{\partial n}\Big|_{n=0} = 0 \quad \& \quad \frac{\partial \boldsymbol{R}_n}{\partial n}\Big|_{n=N} = 0.$$
(2.8)

Also, the moments of the random forces are now given as:

$$\langle f_n(t) \rangle = 0 \quad \& \quad \langle f_{n\alpha}(t) f'_{m\beta}(t') \rangle = 2\zeta k_B T \delta(n-m) \delta_{\alpha\beta} \delta(t-t').$$
(2.9)

Equations 2.7, 2.8, and 2.9 defined the continuous Rouse model. The Rouse model displays the general features of any model that assumes local interaction. The Rouse model represents the long-time-scale behavior of the local jump model one in the same way as the Gaussian chain represents the large large-scale properties of a polymer with only short-range interaction.

Eq. 2.7 represents a Brownian motion motion of a couple of oscillators. A standard way of training such a system is to find the normal independent coordinates. In terms of the coordinates X_p , defined by,

$$\boldsymbol{X}_{p} \equiv \frac{1}{N} \int_{0}^{N} dn \cos\left(\frac{p\pi n}{N}\right) \boldsymbol{R}_{n}(t) \text{ with } p = 0, 1, 2, ...,$$
(2.10)

equation 2.4 can be rewritten as

$$\zeta_p \frac{\partial}{\partial t} \boldsymbol{X}_p = -k_p \boldsymbol{X}_p + \boldsymbol{f}_p, \qquad (2.11)$$

where,

$$\zeta_0 = N\zeta \& \zeta_p = 2N\zeta \text{ for } p = 1, 2, ...,$$
 (2.12)

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$$k_p = \frac{2\pi^2 k p^2}{N} = \frac{6\pi^2 k_B T}{N b^2} p^2 \text{ for } p = 0, 1, 2, ...,$$
(2.13)

and the f_{ρ} 's are the random forces which satisfy

$$\langle f_{\alpha\beta} \rangle = 0, \langle f_{p\alpha}(t) f'_{q\beta}(t') \rangle = 2\delta_{pq}\delta_{\alpha\beta}\zeta_p k_B T \delta(t-t').$$
(2.14)

Since, the random forces are independent of each other, the motions of the X_p 's are also independent. Thus the motion of the polymer is decomposed into independent modes. The normal coordinate X_p with p > 0 represents the internal conformation of the polymer. Consider for example the end-to-end vector, $P(t) \equiv R_N(t) - R_0(t)$, which is expressed by X_p as,

$$\mathbf{P}(t) = -4 \sum_{p:odd \ integer} \mathbf{X}_p(t).$$
(2.15)

One may calculate the time correlation function as:

$$\langle \mathbf{P}(t) \cdot \mathbf{P}(0) \rangle = Nb^2 \sum_{p=1,3,\dots} \frac{8}{p^2 \pi^2} \exp(-\frac{tp^2}{\tau_1}) \text{ with } \tau_1 = \frac{\zeta_1}{k_1} = \frac{\zeta N^2 b^2}{3\pi^2 k_B T}.$$
 (2.16)

The last equation indicates that the first mode X_1 , mainly governs the movement of the end-toend vector. In general, X_p represents the local motion of the chain, which includes N/p segments and corresponds to the motion with the length scale of the order $[Nb^2)/p]^{1/2}$.

The rotational relaxation time τ_r of a polymer can be defined by the longest relaxation time of the correlation function $\langle P(t) \cdot P(0) \rangle$:

$$\langle \mathbf{P}(t) \cdot \mathbf{P}(0) \rangle \propto \exp(-\frac{t}{\tau_r}) \text{ for } t \gtrsim \tau_r.$$
 (2.17)

Since *N* is proportional to the molecular weight *M*, it is possible to indicate the molecular weight dependence for τ_r and self-diffusion constant of the center of mass, D_G as:

$$D_G = \frac{k_B T}{N\zeta} \propto M^{-1} \quad \& \quad \tau_r = \frac{\zeta N^2 b^2}{3\pi^2 k_B T} \propto M^2.$$
(2.18)

Here, D_G is defined as $D_G = \lim_{t\to\infty} \frac{1}{6t} \langle (\mathbf{R}_G(t) - \mathbf{R}_G(0))^2 \rangle$, where $\mathbf{R}_G \equiv \frac{1}{N} \int_0^N dn \, \mathbf{R}_n = \mathbf{X}_0$ (\mathbf{X}_0 represents the position of the center of mass). The Rouse model is conceptually quite important; nevertheless, it is inappropriate for dilute polymer solutions because it neglects the hydrodynamics interactions. However, it has also turned to be a useful model for the dynamics of polymers in melts.

The Zimm model in Θ conditions. To describe the dynamics of linear chain with Gaussian chain statistics in dilute solutions, taking into account the average hydrodynamics interactions one may express:

$$\boldsymbol{H}_{nm} = \frac{1}{8\pi\eta_s |\boldsymbol{r}_{nm}|} [\boldsymbol{\hat{r}}_{nm} \boldsymbol{\hat{r}}_{nm} + I] \text{ for } n \neq m$$
(2.19)

where $\mathbf{r}_{nm} \equiv \mathbf{R}_n - \mathbf{R}_m$ and $\hat{\mathbf{r}}_{nm}$ is the unit vector in the direction of \mathbf{r}_{nm} . Considering the Eq. 2.19, the Langevin equation (see Eq. 2.1) change to,

$$\frac{\partial}{\partial t}\boldsymbol{R}_{n} = \sum_{m} \boldsymbol{H}_{nm} \cdot \left(-\frac{\partial U}{\partial \boldsymbol{R}_{m}} + \boldsymbol{f}_{m}(t)\right), \qquad (2.20)$$

Transforming the last equation in the continuous limit and into normal coordinates, the Eq. 2.20 change to,

$$\zeta_p \frac{\partial}{\partial t} X_p(t) = -k_p X_p + f_p(t)$$
(2.21)

where,

$$\zeta_p = (12\pi^3)^{1/2} \eta_s (Nb^2 p)^{1/2} \quad \& \quad \zeta_0 = \frac{3}{8} (6\pi^3)^{1/2} \eta_s b \sqrt{N} \text{ for } p = 1, 2, \dots$$
(2.22)

$$k_p = \frac{6\pi^2 k_B T}{Nb^2} p^2$$
 for $p = 0, 1, 2, ...$ (2.23)

Given ζ_p and k_p , the diffusion constant and the relaxation time are obtained as

$$D_G = 0.196 \frac{k_B T}{\eta_s \bar{R}'}$$
(2.24)

$$\tau_p = \tau_1 p^{-3/2}$$
 with $\tau_1 = \tau_r = 0.325 \frac{\eta_s \bar{R}^3}{k_B T}$ (2.25)

where $\bar{R} = \sqrt{N}b$. Equations 2.24 and 2.25 predict the molecular weight dependence of D_G and τ_r as:

$$D_G \propto M^{-1/2} \& \tau_r \propto M^{3/2}$$
 (2.26)

which agrees with experimental results [5]. This model describe a chain immersed in a poor solvent, sometimes called Θ solvent condicion, where to cancel the effects of exclude volume.

Zimm model in a good solvent. So far, we have been considering the Θ conditions; the excluded volume interactions are taken into account if we add a potential,

$$U_1 = \frac{1}{2} \nu k_B T \sum_{n,m} \delta(\boldsymbol{R}_n - \boldsymbol{R}_m), \qquad (2.27)$$

where v is the excluded volume. If such potential is introduced, the Langevin equation becomes nonlinear; however, it is possible to treat the problem in a simple way by assuming the linear behavior,

$$\zeta_p \frac{\partial}{\partial t} \boldsymbol{X}_p(t) = -k_p \boldsymbol{X}_p + \boldsymbol{f}_p(t)$$
(2.28)

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and include the excluded volume effect in the parameters ζ_p and k_p . The resolution of Eq. 2.28 is quite difficult and may be seen in detail in [5]. The parameters D_G and τ_r for this model are:

$$D_G \approx \frac{k_B T}{\eta_s N^{\nu} b} \quad \& \quad \tau_r \approx \frac{\eta_s N^{3\nu} b^3}{k_B T}$$
(2.29)

where ν is a parameter that senses the interaction between two segments in the polymer chain. Using $R_g \approx N^{\nu}b$, Eq. 2.29 can be written in the same form as Eq. 2.26,

$$D_G \approx \frac{k_B T}{\eta_s R_g} \& \tau_r \approx \frac{\eta_s}{k_B T} R_g^3.$$
 (2.30)

These results are the same as for rigid spheres [5]: the characteristic behavior of the dilute solution is quite similar to the suspension of spheres of radius R_g . This model represents a chain in which the interaction between the polymer segments and the solvent molecules is energetically favorable, which will cause the polymer chain to expand. This condition is sometimes referred to as good solvent condition.

Equations 2.18, 2.26, and 2.30 represent the mesoscopic parameters, D_G and τ_r , for different models assuming different hypotheses concerning the interaction between polymer chains and the medium. At the mesoscopic level, the interaction between chains and the medium is intimately related to viscoelasticity parameters, *e.g.*, intrinsic viscosity [η] and complex modulus $G^*(\omega) = G'(\omega) + iG''(\omega)$; in the next section will mentioned in detail about $G^*(\omega)$. For example, the intrinsic viscosity can be evaluated as:

$$[\eta] = \frac{N_A k_B T}{M \eta_s} \sum_{p=1}^{\infty} \frac{\zeta_p}{2k_p}.$$
(2.31)

The sum is evaluated for various models:

i) The Rouse model:

$$[\eta] = \frac{N_A}{M\eta_s} \frac{N^2 b^2 \zeta}{6\pi^2} \sum_{p=1}^{\infty} \frac{1}{p^2} = \frac{N_A}{M\eta_s} \frac{N^2 b^2 \zeta}{36}.$$
(2.32)

ii) The Zimm model for Θ solvent:

$$[\eta] = \frac{N_A}{M} \frac{\left(\sqrt{N}b\right)^3}{\sqrt{12\pi}} \sum_{p=1}^{\infty} p^{-1.5} = \frac{N_A}{M} 0.425 \left(\sqrt{N}b\right)^3.$$
(2.33)

iii) The Zimm model for good solvent:

$$[\eta] = \frac{N_A}{M} N^{3\nu} \sum_{p=1}^{\infty} p^{-3\nu} b^3 = \frac{N_A}{M} N^{3\nu} b^3.$$
(2.34)

Writing the molecular weight dependence of [η], [η] $\propto M^{\nu_{\eta}}$, as:

$$\nu_{\eta} = \begin{cases} 1 & Rouse \ model \ (ideal \ chain \ in \ vacuum), \\ 0.5 & Zimm \ model \ (\Theta \ solvent), \\ 3\nu - 1 = 0.8 & Zimm \ model \ (good \ solvent). \end{cases}$$
(2.35)

The viscoelasticity moduli can be calculated in the same way as the intrinsic viscosity; its functional forms are:

$$G'(\omega) = \int_0^\infty dt\omega \sin(\omega t) \sum_p \exp\left(-\frac{t}{\tau_p}\right) = \sum_{p=1}^\infty \frac{(\omega\tau_p)^2}{1+(\omega\tau_p)^2},$$
(2.36)

$$G''(\omega) = \int_0^\infty dt\omega \cos(\omega t) \sum_p \exp\left(-\frac{t}{\tau_p}\right) = \sum_{p=1}^\infty \frac{(\omega\tau_p)}{1 + (\omega\tau_p)^2}.$$
(2.37)

Where the relaxation time τ_p can be written as:

$$\tau_p = \frac{\zeta_p}{2k_p} = \tau_1 p^{-\mu}$$
, where (2.38)

$$\mu = \begin{cases} 2 & Rouse model (ideal chain in vacuum), \\ 3/2 & Zimm model (\Theta solvent), \\ 3v & Zimm model (good solvent). \end{cases}$$

The expressions for viscoelasticity moduli are simplified in two cases.

i) $\omega \tau_1 \ll 1$: In this case, $G'(\omega)$ and $G''(\omega)$ are approximated as

$$G'(\omega) = (\omega\tau_1)^2 \sum_{p=1}^{\infty} p^{-2\mu},$$
(2.39)

$$G''(\omega) = \omega \tau_1 \sum_{p=1}^{\infty} p^{-\mu}.$$
 (2.40)

Hence, $G'(\omega)$ and $G''(\omega)$ are proportional to ω^2 and ω , respectively.

ii) $\omega \tau_1 \gg 1$: In this case, the sum over p can be replaced by an integral so that:

$$G'(\omega) = (\omega \tau_1)^{1/\mu} \frac{\pi}{2\mu \sin(\pi/2\mu)},$$
(2.41)

$$G''(\omega) = (\omega \tau_1)^{1/\mu} \frac{\pi}{2\mu \cos(\pi/2\mu)}.$$
 (2.42)

For different cases, can be shown that:

Rouse model ($\mu = 2$):

$$G'(\omega) = 1.11(\omega\tau_1)^{1/2} \& G''(\omega) = 1.11(\omega\tau_1)^{1/2}.$$

Zimm model in Θ solvent ($\mu = 3/2$):

$$G'(\omega) = 1.21(\omega\tau_1)^{2/3} \& G''(\omega) = 2.09(\omega\tau_1)^{2/3}$$

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(2.43)

(2.44)

Zimm model in Θ solvent ($\mu = 9/5$):

 $G'(\omega) = 1.14(\omega\tau_1)^{5/9} \& G''(\omega) = 1.38(\omega\tau_1)^{5/9}$

The last three equations may be interpreted as a bridge between rheological behavior and the relaxation dynamics of polymeric chains. For example, if the complex modulus scale as $|G^*(\omega)| \sim \omega^{5/9}$ at high frequency, the dynamic of polymer chains corresponds to Zimm modes in Θ solvent. In this work, Zimm modes in Θ solvent are sometimes called Rouse-Zimm modes because the model captures the hypotheses of both models.

At higher polymer concentrations, the presence of other chains will affect its dynamics. To explain chain relaxation, Doi and Edwards developed the reptation model proposed by de Gennes [44]. Relaxation gradually uncouplies each chain that diffuses along its contour, simulating a tube. The tube is formed with neighboring chains representing a set of obstacles normal to the contour of the chain. Finally, each chain repels out of its original tube, and a new tube is created at equilibrium where there is no more stress. In the following sections, we show a diagram to explain the reptation model (see Fig. 2.4). The reptation of the chain through its tube modifies the viscoelasticity of the system, *i.e.*, the Rouse dynamics is stopped by the tube constrictions, and the relaxation modulus is proportional to the fraction $\psi(t)$ of the polymer that is confined in the tube, then:

$$G(t) = G_0 \psi(t) = G_0 \sum_{p;impar} \frac{8}{p^2 \pi^2} \exp\left(\frac{-p^2 t}{\tau_R}\right),$$
(2.46)

where τ_R is the relaxation time. At high frequencies, the polymer dynamics follows the Rouse model, and at short frequencies, the reptation dynamics is manifested. Furthermore, Cates [60] found that the relaxation modulus can be approximated as $G(t) \sim \exp\left(-\frac{t}{\tau_R}\right)^{1/4}$, with τ_R a relaxation time with the contribution of multiple times.

2.1.2 Semi-flexible polymers

Morse proposed a model to predict the viscoelastic properties of semi-flexible polymers [45–47]. He considers a "*tightly–entangled*" solution in which the mesh size is shorter than the persistence length ($\xi \ll l_p$), and each polymer is confined to a diameter tube shorter than l_p . The dynamic of the polymer chain is described by the reptation model analogous to the Doi-Edwars

(2.45)

model [5], where: i) Each polymer is confined to a weakly-curvature tube, ii) tangential motion of the polymer relative to the tube is resisted only by viscous forces, and iii) the tube deforms affinely. Under last consideration, the intermolecular stress can be discretized in three terms:

- i) The curvature stress, which contains both purely mechanical bending energy, and entropic contributions arising from the orientational entropy of the links. It can be proven that any bending joint exactly vanishes when the curvature in that joint is locally equilibrated.
- ii) The orientational entropy, which is a residual contribution of the link orientational entropy from the two end links.
- iii) The tensorial stress that arises from the link tension.

The model predicts that the relaxation of individual polymer chains controls entirely the shear modulus at high frequencies. When a polymer network is shared, the thermal modulation of the stifness polymer section is either compressed or stretched, depending on their orientation concerning the shear direction. Since the filaments are practically inextensible along their contour, the chain conformations rethermalize by a redistribution of their bending modes, leading to a characteristic scaling of the polymer contribution to the shear modulus as $G^*(\omega) \propto \omega^{3/4}$:

$$G^*(\omega) \approx \frac{1}{15} \rho \kappa l_p (-2i\zeta/\kappa)^{3/4} \omega^{3/4} - i\omega\eta, \qquad (2.47)$$

where ρ is the density, κ is the bending stiffness ($\kappa = l_p/(k_BT)$), and ζ is the lateral drag coefficient per unit length [48].

2.2 Living polymers: Wormlike micelles solutions

The last section discussed the dynamic of polymer systems and different assumptions about the relaxation process, *e.g.*, in Rouse-Zimm modes, and bending modes. One of the most important hypotheses is that the polymer chains do not change their structure, length, or topology within experimental timescales; here, the polymer chain is the same. However, when the polymer chain does not obey the last constraints, the system formed for these polymer chains is called "living polymer." Living polymers are different from their non-living counterparts as random architectural changes such as break, fusion, and recombination alter the initial architecture of polymers on timescales similar to their relaxation time [6]. The following will discuss the structure and dynamics of wormlike micelles solutions (WLMs) as an example of a living polymer; WLMs share the same mesoscopic scales and relaxation mechanisms of polymer chains, in addition to the process of breakage and recombination.

2.2.1 Structure and Relaxation Dynamics

Surfactants are amphiphilic molecules consisting of a non-polar hydrophobic portion attached to a polar or ionic portion (hydrophilic part). The hydrophilic portion may be nonionic, ionic, or zwitterionic, and in the latter two cases, accompanied by the counterion. In aqueous media, the hydrocarbon chain interacts weakly with water molecules. In contrast, the polar head interacts strongly with water through ion-dipole interactions, adopting most possible conformations and maximizing entropy. The balance between the hydrophobic and hydrophilic parts in the amphiphile confers exciting properties, *e.g.*, accumulation in the interface or their association with forming aggregates called micelles [49]. At low surfactant concentrations, many dissolution properties are similar to electrolyte solutions except for lowering surface tension, which decreases with increasing concentration. However, as the concentration increases, the solution properties change for a particular critical micelle concentration (CMC). Above the CMC, the amphiphilic molecules associated in micelles with the former aggregates are generally spherical, and the surface tension remains constant with increasing concentration [49].

For micelles embedded in a polar solvent, the hydrophobic groups are directed towards the interior of the aggregate and the polar groups towards the solvent, thus reducing the contact between the hydrocarbon chain and the water and decreasing the system's free energy. The morphology of these aggregates can vary in shape and size, and their final structure depends on different parameters such as geometric constraints imposed by the surfactant, concentration, ionic strength of the solution, temperature, etc. [7]. The forces that determine the association of surfactants in defined structures, and hence their curvature, are hydrophobic effects (when chains are brought together) and repulsion between polar groups (of electrostatic origin for ionic groups and steric for non-ionic groups) resulting in geometrical parameters. These considerations are represented by the geometrical parameter *p*:

$$p = \frac{V}{a_0 l_c},\tag{2.48}$$

where *V* is the volume of the hydrophobic chain, l_c is the effective chain length, and a_0 is the surface area of the polar head. Figure 2.2a shows these characteristic lengths and interactions between surfactant molecules. a_0 is the most significant factor determining the competition between the interaction between hydrophilic and hydrophobic parts of the amphiphilic. Israelachvili [50]showed that spherical micelles are obtained with p < 1/3; for 1/3 obtained non-spherical micelles may be obtained (e.g., cylindrical micelles); for <math>1/2 a flat micellar system is achieved, and with <math>p > 1 inverse micelles (e.g., water in oil). One intriguing aspect of surfactant behavior is its adaptability. For instance, the curvature of micelles can be altered by modifying the molecular structure, which in turn changes the value of a_0 . For example, increasing the ionic concentration reduces the electrostatic repulsion between the charged groups by shielding, so, a_0 decreases, promoting the transition from spherical to cylindrical micelles [51].



Figure 2.2. a) Micelle formation according to the geometric model from [51]; the amphiphilic lengths show in the imagen. b) WLMs formation according to amphiphilic concentration from [52]; here, can be appreciated different regimens concentration.

Wormlike micelles (WLMs) formation occurs when spherical micelles fuse to form a cylindrical, elongated, and uniform aggregate, thus conferring two very important characteristics: an elongated cylindrical shape and flexibility [7]. One way to induce the transition to WLMs is by increasing the surfactant concentration above the CMC, promoting the uniaxial growth of the micelles to cylindrical [53]; Figure 2.2.b shows WLM formation with increasing amphiphilic concentration. The packing of amphiphiles into elongated cylindrical micelles is determined by the entropy/energy balance. Energy is minimized when the curvature is uniform throughout the formation of infinitely long linear structures. However, the system's entropy introduces a degree

of randomness through the bending of the cylinders and by topological defects in the form of end caps or chain attachment points. If the scission energy of a WLM (the energy required for creating two end caps from an infinite cylinder) is sufficiently large, then the semi-flexible linear micelles may become very long and entangled. On the other hand, end caps increase entropy by increasing the number of micelles in the system. As a result, although the occurrence of topological defects is due to entropy, the spontaneous curvature of the amphiphile establishes the type of defect that determines the system's structure. Also, branch junction points increase the number of possible configurations and entropy, enable percolation, and form extended micellar networks, leading to a multi-connected rather than an entangled network of WLMs.

As with flexible polymers, three important lengths relate to the flexibility and mobility of these systems: cross-section radius r_c , persistence length l_p , and the contour length L_c , which are shown in Figure 2.3. If amphiphilic concentration increases, a network distinguishes two new lengths: the mesh size ξ , and entanglement length l_e . The cross-section radius is associated with the amphiphilic chain length of amphiphilic; r_c is independent of concentration and order of a few nanometers [55]. The contour length is determined by the surfactant structure, with values from hundreds of nanometers to micrometers [55]; contrary to r_c , L_c depends on the fraction volume of surfactant volume, φ . When micelles are strongly screened or neutral, it can be shown, with a mean-field approach, that the variation of L_c with φ obeys a power law $L_c \approx \varphi^{1/2} \exp(E_c/2k_BT)$, with E_c energy incision of chain. The persistence length l_p is related to the elastic modulus of curvature κ as $l_p \approx \kappa/(k_BT)$; it may understood as the rigid section of the cylindrical body, and usually with values of a few tens of nanometers [55]. Also, l_p depends on the local environment, such as charged surfactant groups, the hydrocarbon chain length, and the dependence on control parameters, *e.g.*, ionic strength, temperature, etc. [7]. Figure 2.3 shows the mesoscopic characteristic lengths of WLM in a semidilute regimen.



Figure 2.3. Mesoscopic structure of wormlike micelles. Image from [56].

With respect to the dynamic relaxation processes of WLMs, after applying shear strain to a micellar solution, the stress accumulated in the network must be released through a relaxation mechanism, breaking and recombination process, and the reptation of micellar chains. The reptation model for polymers was proposed by Doi and Edwards [5]. The reptation process consists of a gradual uncoupling of some chains, diffusing along its own contour, which is a tube simulation. This tube is formed with neighboring chains, representing a set of obstacles normal to the contour of the chain. Eventually, each chain scapes from its original tube, and a new tube is created at equilibrium, with no more stress [8,57]. Cates in the late 80's [8,57] proposed a relaxation model for living polymers (Figure 1.3) that describes the viscoelastic behavior (in a linear regime), including in the reptation model the breaking-recombination mechanism. Here, distinguishing two times, reptation time τ_{rep} , and the breaking-recombination time τ_b with $\tau_b \ll$ τ_{rep} ; the chains break and recombine frequently before the chain disengages from its tube by reptation. Initially, a tube of length *x* is considered, and away from the tube boundaries, the chain breaks within a distance, traversed by reptation, of λ along *x*. This new segment diffuses and combines with a new chain recombining, creating a new tube that, at equilibrium, is stressless.



Figure 2.4. In the left-hand reptation Doi-Edwards's model, and on the right-hand Cates' reptation model. For reptation model: a) the chain is inside its tube, b) and c) the chain diffuses and d) the chain is completely unhooked from its tube. In the Cates model: a) The chain has crawled a length λ , b) chain breakage occurs, c) the chain diffuses through the tube and c) the chain recombines. Image from [8].

Considering the Cates relaxation model, it is obtained that the stress decays exponentially and can be described by a simple exponential function:

$$\sigma(t,\dot{\gamma}) = \sigma_0(\dot{\gamma}) \exp\left(-\frac{t}{\tau_R}\right),\tag{2.49}$$

where τ_R is the relaxation time and equal to the geometric mean of τ_{rep} and τ_b , *i.e.*, $\tau = \sqrt{\tau_b \cdot \tau_{rep}}$. Mono exponential decay motives the description of the linear viscoelasticity of WLMs solutions by two parameters: the elastic modulus G_0 and the relaxation time defined as $\tau_R = \eta_0/G_0$; both quantities can be extracted from the linear viscoelasticity spectra [8].

Inherently, there is a relationship between mesoscopic structure and relaxation dynamics of micellar chains; this relationship may help obtain mesoscopic length values of micellar networks. As a summary: At time scales shorter than WLM breaking-recombination time, corresponding to high frequencies, the Maxwellian stress relaxation processes are fundamentally frozen; here, the WLM chain behaves like flexible polymer chains. So, the stress relaxes according to flexible polymer, first dominated by the Rouse-Zimm modes and at higher frequencies by internal relaxation of Kuhn segments. As mentioned above, $G^*(\omega)$ exhibits a power-law behavior, $|G^*| \sim \omega^{\nu}$ in both regimens; for the Rouse-Zimm regime with an exponent $\nu \sim 5/9$, which shifts to $\nu \sim 3/4$, where the internal bending modes of Kuhn segments dominate. That change occurs at a frequency ω_0 , which is related to the persistence lengths as [58]:

$$\omega_0 \approx \frac{k_B T}{8\eta_s l_p^3},\tag{2.50}$$

where η_s is the solvent viscosity. De Gennes [59]proposed the relationship with scaling arguments; here, the elastic blob ξ is a characteristic size of elastically effective chains where G_0 is written as the product of the number density of elastic blobs ρ_{el} , as $G_0 = \rho_{el}k_BT$ with the size of an elastic blob (the mesh size) is $\xi = \rho_{el}^{-1/3}$. However, this model does not consider the reptation model which has included a recent correction by Zou & Larson [16]:

$$\xi \cong \left(A \cdot \frac{k_B T}{G_0}\right)^{1/3} \text{ with } A = 9.75$$
(2.51)

In the same way, *le* can be calculated using a relationships proposed Grenek & Cates [60]:

$$l_e = \frac{\xi^{5/3}}{l_p^{2/3}}.$$
 (2.52)

Finally, the contour length can be estimated by incorporating breathing and high-frequency Rouse modes. The exponent in this equation is a correction given by Granek [61]:

$$\frac{G_{min}^{\prime\prime}}{G_0} \cong \left(\frac{l_e}{L_c}\right)^{0.8},\tag{2.53}$$

where G''_{min} is a local minimum of $G''(\omega)$ after the first crossing between $G'(\omega)$ and $G''(\omega)$. The following section shows a summary methodology for obtaining G_0 , $G''(\omega)$, and ω_0 , experimentally and, consequently, the characteristic mesoscopic lengths.

2.2.2 Shear banding phenomena and inertia-elastic instabilities

As the concentration of amphiphilic increases above c^* , a transition similar to the first order transition occurs, from an isotropic disordered phase to an oriented nematic phase, which is typical of liquid crystals [62]. The phase diagram depends on the surfactant, salt concentration, and temperature. Figure 2.5 shows the phase diagram showing the isotropic and nematic transition.



Figure 2.5. Phase diagram corresponding to a CPCI-Hex WLMs solution. The quantity $\langle d \rangle$ refers to the average distance between neighborhoods micelles and q^* the scattering vector in a light scattering experiment. Image from [62].

A similar transition from isotropic to nematic state can be seen when a shear strain is applied to a WLMs solution; an intrinsic tendency to align the chains with each other can be enhanced by disturbing the equilibrium between the micelles' isotropic to nematic phase transition [63]. Above a shear strain, the homogeneous flow becomes unstable because of the alignment of the chains. Then, the sample separates into macroscopic bands that coexist with different viscosities and structures [64]. This phenomenon is called shear banding and has been widely studied [9–13,17,62–65]; the separation occurs along the velocity gradient. The typical flow curve of a system exhibiting shear banding is shown in Figure 2.5.c. When $d\sigma/d\dot{\gamma} < 0$, the flow is homogeneous and unsteady [65]; this triggers the formation of bands that coexist between two shear velocities and at constant stress. The isotropic-nematic transition obeys the lever rule, with $\dot{\gamma}_1 < \dot{\gamma} < \dot{\gamma}_2$:

$$\dot{\gamma} = \dot{\gamma}_1 (1 - \alpha) + \dot{\gamma}_2 \alpha, \qquad (2.54)$$

where α is the volume fraction corresponding to the induced phase shear strain. The last equation is valid in a simple scenario where only two single bands coexist: the isotropic and nematic bands.



Figure 2.5. Isotropic - nematic transition for a TC geometry. a) Homogeneous flow, b) inhomogeneous flow where isotropic and nematic phases coexist between them, and c) typical flow curve. Image from [66].

For a weak flow, the measurement geometry defines the velocity profile of the flow, where flow symmetry breaks when the flow is strong enough to eventually become turbulent. The understanding of instabilities is primarily attributed to the compression of the Concentric Cylinder system, often called Taylor-Cuette (TC geometry) [18]. In the case of Newtonian fluids, such as water, the original study by Reynolds in 1883 takes inertia as the parameter that determines instabilities. The Reynolds number, a crucial factor in fluid dynamics, controls this parameter. The Reynolds number, defined as $Re \equiv (dU)/v$, where d is a characteristic length along the velocity gradient, U is a characteristic velocity, and v the kinematic viscosity defined by $v = n/\rho$ with ρ denoting the density. Re number is frequently interpreted as the ratio of inertial destabilizing forces to viscous stabilizing forces.

Due to inertia in a Newtonian fluid, the Reynolds number can be rewritten in terms of a characteristic time (called sometimes viscous diffusive time) τ_i as $Re = \tau_i \dot{\gamma}$ where $\tau_i = d^2/v$. In viscoelastic fluids, the relaxation upon perturbation brings an additional characteristic time τ_R due to the material's internal structure [18]. For example, in polymeric or micellar solutions, the internal structure can be altered by the imposition of a flow, and such structural change, in turn, can modify the flow [19]. It has been known since 1990 that flow instabilities can occur in complex fluids at low Reynolds [15,67]. For example, with experiments on polymer solutions and linear stability analysis of viscoelastic models, Learson [67] showed the existence of a purely elastic instability mechanism for a Cuette flow.

In the small gap limit, instabilities are driven by the Weisenberg number, defined as $Wi = \tau_R \dot{\gamma}$ with τ_R a viscoelastic relaxation time. This relaxation time, a key parameter in viscoelastic fluid dynamics, is crucial in driving instabilities. The quantity driving the instabilities is the Taylor number, $Ta = \Lambda^{1/2}Wi$. In polymeric fluids, τ_R can be the Rauss-Zimm time or reptation relaxation

time, depending on the cross-linking of the chains [19]. Learson concluded that vortex flow emerges when $Ta > Ta_c$, e.g., for a viscoelastic fluid described by the upper convective Maxwell model, one has a $Ta_c \approx 6$ [67]. When the Reynolds and Weisenberg numbers are large, the instabilities are called elastic-inertial instabilities. Dimensional analysis suggests that the Taylor number regarding Re and Wi can be written as $Ta = \Lambda^{1/2} f(Re, Wi)$. The function f(Re, Wi) satisfies that $\lim_{El\to 0} f(Re, Wi) = Re$, and $\lim_{El\to\infty} f(Re, Wi) = Wi$ where El is the elastic number defined as:

$$El \equiv \frac{Wi}{Re} = \frac{\tau_R}{\tau_i},\tag{2.55}$$

here, *El* number drives the inertio-elastic instabilities. Figure 2.6 shows the elastic-inertial transitions for a WLMs solution without shear banding as a function of shear rate. For *El* \sim 1, micellar fluid undergoes a viscoelasticity transition called Elastically Dominated Turbulence (EDT), which is modified by the system's elasticity concerning a Newtonian fluid; this is an example of elastic-inertia instabilities in complex fluids which phenomenology is not yet fully understood [25].



Figure 2.6. Transition of inertio-elastic instabilities in a TC geometry of WLMs solution ([CTAB] = 0.1 M and [NaNO3] = 0.3 M at 30 °C). The nomenclature is: SV = Standing Vortex, DRSW = Disordered Rotating Standing Wave, EDT =Elastically Dominated Turbulence. Imagen from [19], which construction can be seen graphically in the supplementary material.

2.3 Polymer gels

A gel can be obtained from the covalent or attractive bonding between polymeric chains in solution, as exemplified in Figure 2.7. In solution, called a sol, the bonding of adjacent polymeric chains (junction points) can occur, forming progressively larger branched polymers. If the crosslinking continues, larger branched polymers will still be obtained, and the molecule appears to encompass the entire system. If the crosslinking process continues, an infinite polymer is formed, forming a structure called a gel. The transition from a system that contains finite chains to a system containing infinite chains is called the sol-gel transition, and the critical point where the gel first appears is called the gel point [43]. Figure 2.7 shows a vignette of the formation of an infinite gel network.



Figure 2.7. Linking points formation between flexible polymeric chains: a) four linking points, b) eight linking points, c) 10 linking points. Larger branched polymers are highlighted, and the tenth crosslinking point (in black) forms a single gel. Imagen from [43].

During the gelation process, crosslinking can occur by changing thermodynamic conditions such as temperature, pH, polymer concentration, or adding salts. The networks are held together in these gels by electrostatic interactions, hydrophobic or van der Waals interactions, or combinations of these, categorized as physical gels. There are also gels cross-linked by chemical bonds, such as *e.g.*, disulfide bonds or reactions mediated by a catalytic agent, as in the case of rubbers, resulting in non-reversible gels categorized as chemical gels [26,68]. Additionally, a combination of these two crosslinking routes, by physical and chemical interaction, makes it possible to form physical-chemical gels.

2.3.1 Gelation percolation model

The following discussion can be seen in detail in the textbook *Polymer Physics* by M. Rubinstein & Colby, R. H. [43]. A bond percolation model can describe the sol-gel transition. In a simple bond percolation model, all lattice sites are assumed to be occupied by monomers. The chemical reaction between monomers is modeled by randomly connecting monomers on neighboring sites by bonds. The fraction of all possible bonds formed at any point in the response
is called the extent of reaction, p, which increases from zero to unity as the reaction proceeds. In this model, a polymer is represented by a cluster of monomers (sites) connected by bonds. When all possible bonds are formed, the reaction is completed (p = 1), and the polymers are a fully developed network.

The system undergoes a connectivity transition at the percolation threshold or gel point p_c . Slightly below the gel point, the system is a polymer-dispersed mixture of branched polymers (see Fig. 2.8a). Slightly beyond the gel point, the system is still mainly a polydisperse mixture of branched polymers, but one structure percolates through the entire system (see Fig. 2.8b). This structure is called the *incipient gel*, a weak structure quite different from the fully developed network above the p_c . This connectivity transition from a sol below p_c to a gel permeated with sol above p_c is known as the gelation transition.

At any specified extent of reaction p, the dimensionless number density of molecules with N monomers is n(p, N), defined as the number of N-monomers divided by the total number of monomers. This number density is proportional to the probability that a randomly selected polymer has N monomers, the number fraction of N-monomers $n_N(p)$. The sol fraction is defined as the fraction of all monomers that are either unreacted or belong to finite-size polymers (the sol):

$$P_{sol}(p) = \sum_{N=1}^{\infty} Nn(p, N) = \sum_{N=1}^{\infty} w(p, N).$$
(2.56)

The last sum is only made over the finite-size species, meaning that above the gel point, the gel is excluded. Eq. 2.56 employs the definition of the dimensionless weight density of N-monomers, w(p, N). The weight density w(p, N) is the probability that a randomly chosen monomer is part of a polymer with N monomers and is equal to the weight fraction $w_N(p, N)$ times the sol fraction $P_{sol}(p)$. The gel fraction is defined as the fraction of all monomers belonging to the gel. Every monomer must be either part of the sol or part of the gel, so the sum of the sol and gel fractions is unity:

$$P_{gel}(p) + P_{sol}(p) = 1. (2.57)$$

Below the gel point, all monomers are either unreacted or belong to finite-size polymers, and therefore, the sol fraction is unity, and the gel fraction is zero:

$$P_{sol}(p) = 1, \quad P_{gel}(p) = 0 \quad \text{for } p \le p_c;$$
 (2.58)

above the gel point, the gel fraction is non-zero, and the sol fraction is less than unity.



Figure 2.8. a) and b) Gelation is a bond percolation transition; the percolation cluster is indicated by darker shading. c) Mean-field prediction of sol and gel fraction for functionality f = 3. Imagen from [43].

The gel fraction is the probability that a randomly selected monomer belongs to the gel. The gel fraction is the order parameter for gelation similar to critical phenomena; here, the order parameter is continuous through the transition, which means that gelation is analogous to a continuous phase transition. The growth of the gel fraction is accompanied by a simultaneous decay of the sol fraction beyond the gel point, p_c . Figure 2.8c shows a prediction for pc according consider to mean-field theory as well as the P_{sol} and P_{gel} fractions.

Once the idea of the percolation model has been clarified, a convenient way to discuss the sol-gel transition is to a bond percolation model on a Bethe lattice; here, one assumes that all lattice sites are occupied by monomers and the possible bonds between neighboring monomers are either formed with probability p or left unreacted with probability 1-p. In the simple version, called the random bond percolation model, the probability p of forming each bond is assumed to be independent of any other bonds in the system. The average number of bonds between the sites is p(f - 1); here, f is the functionality (number of neighboring sites capable of doing a link). If this average number of bonds p(f - 1) is less than unity (p < 1/(f - 1)), the structure of the lattice does not survive for long. If this average number of bonds p(f - 1) is greater than unity (p > 1/(f - 1)), the structure of the linking lattice multiplies indefinitely, forming an infinite genealogical branched family tree.

The transition between these two cases is the gel point:

$$p_c = \frac{1}{f - 1} \tag{2.60}$$

Below the gel point (for $p < p_c$) there are only finite-size branched polymers, while above the gel point (for $p > p_c$) there is also at least one infinity polymer (the gel) in addition to many finite-size branched polymers. The distribution of polymer sizes changes with the fraction of formed bonds p. For small extends of reaction $p \ll p_c$ there are only small polymers, while near the gel point some large branched polymers are present. A unique feature of percolation on a Bethe lattice is that there are many infinite polymers present in the same system above the gel point; at p = 1, is present a single infinite network polymer on a fully reacted Bethe lattice.

2.3.2 Chambon & Winter criteria

The gel point marks a transition between liquid-like behavior (sol state) and solid-like behavior (gel state) from viscoelasticity. Returning to part of what was mentioned above during the initial stages of the cross-linking process, branched molecules of a wide distribution of sizes and of several architectures are formed. Their average molecular weight increases with increasing extent of the crosslinking reaction. The system reaches its gel point at a critical $p = p_c$ at which either the weight-average molecular weight diverges to infinity or a first macromolecular cluster extends across the entire sample. Consequently, the system loses solubility, the shear zero viscosity, η_o , diverges to infinity, and the relaxation modulus, G(t), rises to a finite value. The newly formed macroscopic network structure starts to coexist with the remaining branched molecules, which are not yet attached. Beyond the gel point, the network stiffness increases steadily with increasing crosslink density until the system completes the crosslinking reaction.

Winter & Chambon [28] investigated the end-linking reaction of polydimethylsiloxane (PDMS) chains. Divinyl-terminated PDMS was linked with a tetrasilane (f = 4) moiety, and the molecular mass of the prepolymer was below the entanglement limit. Using the time-temperature superposition procedure, they could reconstruct the mechanical spectra over an extensive range of frequencies far from the glass transition temperature. They also stopped the chemical reaction at intermediate times of conversion. They observed that the spectra of viscoelasticity moduli exhibit, at some stage of the conversion, a power-law behavior in the entire measurable radial frequency domain.

Figure 2.9. Shows the experimental data from Chambon & Winter's work [28] on the gelation process for PDMS, in which the reactions have been stopped at intermediate states of conversions. Figure 2.9. shows a critical time t_c , or gel point, where the viscoelastic moduli are parallel, *i.e.*, $G''(\omega, t) \sim G''(\omega, t)$. Before the crossing of the viscoelastic moduli, the behavior is that of a viscous liquid where $G''(\omega, t) > G'(\omega, t)$. After the crossing, in the gel state, the behavior is that of an elastic solid with $G''(\omega, t) < G'(\omega, t)$.

They proposed to define the gel point by the following properties:

$$G'(\omega) \propto \omega^n \quad \text{for} \quad 0 < \omega < \infty$$
 (2.61)

$$G''(\omega) \propto \omega^m \quad \text{for} \quad 0 < \omega < \infty$$
 (2.62)

The Kramers-Kronig relation requires the two exponents *n* and *m* to be equal, so the viscoelasticity moduli are then given by:

$$G'(\omega) = G'' \cot(n\pi/2) = \frac{\pi S}{2\Gamma(n)\sin(n\pi/2)} \omega^n,$$
(2.63)

where *S* is related to the gel strength, n (0 < n < 1) exponent reflects the nature and size distribution at the mesoscopic level, and $\Gamma(n)$ is the Euler gamma function of *n*. One way to observe the solgel transition is by the $tan \delta$ function, which is written as $tan \delta = G''/G' = tan (n\pi/2)$ [34]. In the sol state, $tan \delta$ decreases with frequency, while $tan \delta$ increases with frequency in the gel state. At the gel critical point, $tan \delta$ becomes independent of frequency. In this model, the relaxation modulus has the form $G(t) = \mathcal{F}^{-1}\{(G^*(\omega)/i\omega)\} = St^n$. Chambon & Winter's work investigated three cases:

$$n < 1/2 \quad G''(\omega) < G'(\omega)$$

$$n > 1/2 \quad G''(\omega) > G'(\omega)$$

$$n = 1/2 \quad G''(\omega) = G'(\omega)$$
(2.64)

The Winter & Chambon criteria are based first of all, on the experimental observations these authors made by following an end-linking reaction of primary chains in the absence of any physical association (crystallization, phase separation, etc.), where the precursor polymers were non- entangled. At one particular moment in the reaction, viscoelasticity moduli measured in the linear regime exhibit power-law dependence on the frequency. This indicates that an infinite viscosity at zero frequency is also expected from the percolation model; the authors suggest that both approaches, percolation and mechanical spectrum, lead to the same determination.



Figure 2.9. Frequency dependence of $G'(\omega, t_i)$ and $G''(\omega, t_i)$ at different times during ti gelation. The constants A, a_T , and b_T normalize the frequency and viscoelastic moduli. Imagen from [28].

In the pre-gel state, two important quantities diverge, η_0 and the dominant relaxation time $\tau_{(max, S)}$ of the polymer chains undergoing crosslinking. Both quantities diverge as:

 $\eta_0 \sim \Delta p^{-s}; \ \tau_{max,S} \sim \Delta p^{-\nu_s},$ (2.65) where $\Delta_p = (p - p_c)/p_c$. In the post-gel state, the relaxation modulus grows as the degree of crosslinking increases and, therefore, the ratio time associated with the finite ratio modes (whose number decreases as the crosslinking progresses) evolve as follows:

$$G \sim \Delta p^z; \quad \tau_{max,G} \sim \Delta p^{-\nu_G}.$$
 (2.66)

The critical scaling exponents α , *s*, *z*, *v*_s, and *v*_G are positive constants specific to the system undergoing gelation. The critical exponents fulfill relations with each other, *e.g.*, *v*_s = s/(1-*n*) and *v*_G = z/n for the pre-gel and post-gel states, respectively [27]. De Gennes [59] developed a scaling relation between the scaling coefficients where the dimension of the d-space is included as *z* = 1 + (*d* - 2) α . That there are relations between critical exponents is an argument in favor of the universality of the gelation process.

2.3.3 Fractal dimension of gel

Beyond the gel point, its fractal dimension can characterize the formed gel network. A fractal is a self-similar object characterized by a non-integer dimension called sometimes fractal dimension, d_f. Intuitively, a fractal is a geometric object that is *"enormously complicated"*; no matter how much it is magnified, it will never *"zoom out"* to resemble a Euclidian space. The various notions

of fractal dimensions attempt to quantify this complexity, and they can be seen everywhere in nature, for example, in trees, ferns, the bronchial tree, etc. Fractal curves exhibit an exciting property known as self-similarity. Mathematical examples of self-similarity began with Bolzano's 1830 nowhere differentiable function. They continued through the late 19th and early 20th centuries with the Carnot set, the von Koch curve, and the Sierpinsky gasket [43,69].

Fractals can be divided into two main types: deterministic and statistical (or random) ones. The most important property of deterministic fractals is that their fractal dimension is precisely known. In contrast, statistical fractals are constructed by random processes; the best example of such a fractal is the path of a random walker [69]. An example of a deterministic fractal construction is a Koch curve (see Figure 2.10). It starts from a straight-line section and is divided into three equal subsections. On the top of the middle subsection, an equilateral triangle is drawn erasing its bottom side. Thus, up with four segments of equal length instead of the three original ones (stage 1; the generator), repeating the last procedure for these four segments, it is divided into three equal subsections and the middle subsections with the two opposite sides of equilateral triangles (stage 2). At the end of the second step, a line is obtained with each of the four sections consisting of four smaller subsections; this process can continue along several stages (random generator orientation).

To calculate the dependence of the mass of the triadic Koch curve on the length scale, led is draw circles of diameter 2r equal to the lengths of the segments of two consecutive generations. By circles comparison, drawing around the segments of the consecutive generation of the curve, the radius of the circles changes by the factor of 3 ($r_1 = 3r_2$), while the mass m of the section of the curve inside these circles changes by a factor of 4 ($m_1 = 4m_2$). The fractal dimension is defined as:

$$m \sim r^{d_f} \tag{2.67}$$

The fractal dimensions for a triadic Koch curve can be determined from the different ways to calculate m_1 in terms of r_2 ,

$$m_1 = Ar_1^{d_f} = A(3r_2)^{d_f}$$
(2.68)
$$m_1 = 4m_2 = 4Ar_2^{d_f}$$

where A is the proportionality constant. The last equations require that:

$$(3r_2)^{d_f} = 4r_2^{d_f} \implies d_f = \frac{\log(4)}{\log(3)} \cong 1.26.$$
 (2.69)

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Figure 2.10. The first stage is constructing the von Koch curve and a random version of the curve for higher stages. Images modified from [43,70].

Polymers are random fractals, which are examples of regular fractals. For a single conformation of an ideal chain, the mean-square end-to-end distance of an ideal chain is proportional to its degree of polymerization, $N \sim \langle R^2 \rangle$. A similar relation holds for any subsection of the ideal chain with g monomers and size $r, g \sim \langle r^2 \rangle$. Therefore, the fractal dimension of an ideal chain is $d_f = 2$. Polymer fractals are self-similar on a finite, though quite large, range of length scales. There is a natural cutoff of self-similarity on small-length scales, the length I of the bond, and on large scales, the size R of the polymer. The fractal dimension of any polymer is defined through the relation between the number of monomers g in any section of this polymer and the root-mean-square size, $\sqrt{\langle r^2 \rangle}$ of this section:

$$g \sim \left(\sqrt{\langle r^2 \rangle}\right)^{d_f}.$$
 (2.70)

The correlation length ξ for percolation (and gelation) is the size of the characteristic branched polymer with N^* monomers. So, in agreement with Eq. 2.67, the size R and the number of monomers N in a polymer are related by the d_f as $N \sim R^{d_f}$. The same relation is valid for the characteristic branched polymer (network polymer gel):

$$N^* \sim \xi^{d_f}.\tag{2.71}$$

In the gel point, the correlation length must diverge as:

$$\xi \sim (N^*)^{1/d_f}$$
 (2.72)

Fractal analysis has been used to study of the microstructure of biomacromolecules, such as proteins, carbohydrate polysaccharides, and DNA [70,71]. Several methods have been used to study the fractal structure and determine its dimension. These methods can generally be classified as direct methods, such as confocal scanning laser microscopy, dynamic light scattering, small angle X-ray scattering, and small angle neutron scattering. The following section will discuss an indirect method for estimating this quantity.

3. Experimental techniques

3.1 Rheology

Complex fluids have rheological behavior between simple liquids and ordinary solids. So, it is necessary to include the viscosity, η , and the elastic modulus, G_0 , to describe the mechanical behavior through a constitutive equation (relationship between shear stress, σ , and strain, γ). One way to exemplify the mechanical behavior of any material is by a relaxation experiment (see Fig. 3.1); the material is subjected to constant strain for some time, and then the time evolution of the stress is observed once the deformation is released. If the material is a Hooke's solid, the stress remains constant, and the constitutive equation is $\sigma(t) = G_0 \gamma$. On the contrary, if the stress is released instantaneously, it is a Newtonian fluid whose constitutive equation is $\sigma(t) = \eta \dot{\gamma}$, where the shear rate is $\dot{\gamma} = d\gamma/dt$. In an intermediate behavior, the stress is released between a Hooke's solid and a Newtonian fluid, which is said to be viscoelastic behavior [72].

Figure 3.1. Mechanical behavior of a material in a relaxation experiment.

One way to describe the viscoelastic behavior is to consider the history of the material, *i.e.*, the dependence of the stress (or strain) at time t caused by the imposition of a strain (or stress) at a previous time t'. The memory kernel that relates the stress due to a strain is the relaxation modulus for a relaxation experiment, G(t). Additionally, the strain associated with stress is related to compliance for a creep experiment, J(t). In the linear regime, under any small strain (or stress), the response in the stress (or strain) is written according to the superposition principle [73]:

$$\sigma(t) = \int_{-\infty}^{t} G(t - t') \left(\frac{d\gamma}{dt'}\right) dt' \text{ for relaxation experiment,}$$
(3.1)

$$\gamma(t) = \int_{-\infty}^{t} J(t - t') \left(\frac{d\sigma}{dt'}\right) dt' \text{ for creep experiment.}$$
(3.2)

At higher strain (or stress), the stress (or strain) does not depend linearly with γ (or σ), and in this case, equations 3.1 and 3.2 are not valid, being called nonlinear viscoelasticity. The following will discuss linear and nonlinear viscoelasticity in more detail.

3.1.1 Linear viscoelasticity

The linear viscoelasticity spectrum can be obtained from a dynamic approach by measuring the response due to a non-constant signal. The most common measurement protocol is small amplitude oscillatory shear (SAOS); in this case, the signal is a small sinusoidal shear strain, $\gamma(t) =$ $\gamma_0 \sin(\omega t)$ (γ_0 is de amplitude, and ω is the angular frequency), and, as a response, the shear stress. According to the superposition principle for a relaxation experiment (Eq. 3.1), inserting the shear strain and their derivate $\dot{\gamma}(t) = \omega \gamma_0 \cos(\omega t)$, and considering a variable s = t-t', we can follows that:

$$\sigma(t) = \int_0^\infty G(s)\omega\gamma_0 \cos[\omega(t-s)]ds$$

= $\gamma_0 \left[\omega \int_0^\infty G(s)\sin(\omega s)ds \right] \sin(\omega t) + \gamma_0 \left[\omega \int_0^\infty G(s)\cos(\omega s)ds \right] \cos(\omega t)$
= $\gamma_0 [G'(\omega)\sin(\omega t) + G''\cos(\omega t)].$

In the last results, $G'(\omega)$ and $G''(\omega)$ are defined as:

$$G'(\omega) = \omega \int_0^\infty G(s) \sin(\omega s) ds , \qquad (3.3)$$

$$G''(\omega) = \omega \int_0^\infty G(s) \cos(\omega s) ds.$$
(3.4)

Here, $G'(\omega)$ is the component in-phase called storage modulus, or elastic modulus, which is related to the recovery energy of the material after deformation. The variable $G''(\omega)$ is the component out-phase called loss modulus, or viscous modulus, which is associated with the energy dissipated by the flow of the material.

Another way to introduce the viscoelasticity moduli is thinking the response (shear stress) is equal to the signal (shear strain) with a shift phase, δ . So, the shear stress can be written as:

$$\sigma(t) = \sigma_0 \sin(\omega t + \delta)$$

= $\sigma_0 \cos(\delta) \sin(\omega t)$
+ $\sigma_0 \sin(\delta) \cos(\omega t) = \gamma_0 [G'(\omega) \sin(\omega t) + G''(\omega) \cos(\omega t)],$

here, σ_0 is the amplitude of shear stress. The viscoelasticity moduli are defined as:

$$G'(\omega) = \frac{\sigma_0}{\gamma_0} \cos(\delta), \tag{3.5}$$

$$G''(\omega) = \frac{\sigma_0}{\gamma_0} \sin(\delta). \tag{3.6}$$

If the shift phase is $\delta = 0$, the mechanical behavior corresponds to Hooke's solid. On the contrary, if $\delta = \pi/2$, the response corresponds to Newtonian fluid. In a viscoelastic material, the shift phase is $0 < \delta < \pi/2$, *i.e.*, the mechanical behavior is between a solid and an ideal fluid [72].

Considering the viscoelasticity moduli, the complex modulus, $G^*(\omega)$ can be defined as:

$$G^*(\omega) = G'(\omega) + iG''(\omega), \qquad (3.7)$$

where $i^2 = \sqrt{-1}$. The modulus of $G'(\omega)$ is $|G'(\omega)| = \sigma_0 / \gamma_0$ and the shift phase can be correlated with the viscoelasticity moduli as $tan(\delta) = G''(\omega)/G'(\omega)$. Substituting the equations 3.3 and 3.4 in the complex modulus definition (Eq. 3.7) following that:

$$G^*(\omega) = \omega \left\{ \int_0^\infty G(s) \left[\sin(\omega s) + i \cos(\omega s) \right] ds \right\} = \omega \int_0^\infty G(s) e^{i\omega s} ds = i\omega \mathfrak{I}_u \{ G(s) \}.$$
(3.8)

The Eq. 3.8 shows that the complex modulus is equal to the unilateral Fourier transform of the relaxation modulus, *i.e.*, knowing the relaxation modulus of any material is possible to obtain the mechanical behavior of the system. For example, it is possible for Maxwellian fluids to obtain an analytical expression for viscoelastic moduli. Remember that relaxation modulus decays exponentially with a time of relaxation (see Eq. 2.49); the equations 3.3 and 3.4 can be solved with the respect to time as:

$$G'(\omega) = \int_0^\infty \left(\omega \sin(\omega t) G_0 e^{-t/\tau_R}\right) dt = G_0 \frac{(\omega \tau_R)^2}{1 + (\omega \tau_R)^2},\tag{3.5}$$

$$G''(\omega) = \int_0^\infty \left(\omega \cos(\omega t) G_0 e^{-t/\tau_R}\right) dt = G_0 \frac{\omega \tau_R}{1 + (\omega \tau_R)^2}.$$
(3.6)

Interestingly, the complex modulus can be defined into Laplace space through Eq. 3.8 as:

$$G^*(\omega) = s\tilde{G}(s)$$
 using an analytical continuation $s \to i\omega$, (3.9)

where, $\tilde{X}(s) \equiv \int_0^{\infty} X(t)e^{-st}dt$ is the Laplace transform. Getting the unilateral Laplace transform of Eq. 3.1 can be followed that $\tilde{\sigma}(s) = s\tilde{G}(s)\tilde{\gamma}(s)$ [73], where the shear strain applies have a form $\gamma(\omega t) = \gamma_0 e^{i\omega t}$, *i.e.*, a periodic shear strain. Alternatively, for an experiment where the signal is the shear stress in a periodic form $\sigma(\omega t) = \sigma_0 e^{i\omega t}$, called dynamic compliance protocol, can follow that $\tilde{\gamma}(s) = \tilde{J}(s)\tilde{\sigma}(s)$ in the Laplace space (see Eq. 3.2) [73]. Taking the latter expressions, it follows easily that:

$$\tilde{G}(s)\tilde{J}(s) = \frac{1}{s}.$$
(3.9)

The last equation helps obtain compliance by knowing the relaxation modulus or vice versa. By analytical continuation, if $s \rightarrow i\omega$ the equation 3.9 change to $G^*(\omega) = 1/i\omega \tilde{J}(\omega)$ where $J^*(\omega) = i\omega \tilde{J}(\omega)$ is sometimes called the dynamic compliance [73].

3.1.2 Nonlinear viscoelasticity

In the linear viscoelasticity region, the viscoelasticity moduli as a function of shear strain are constant (at $\omega = cte$); this region is called the Linear Viscoelasticity Region (LVR) as shown in Fig. 3.2. where viscoelasticity spectra are the same for all shear strain values. When the shear strain increases, the viscoelasticity moduli are not constant and depend on the shear strain. Fig. 3.2 shows the region of nonlinear viscoelasticity in which the viscoelastic moduli decrease with shear strain; this behavior corresponds to the yield process for microgels [74,75]. However, the decrease of viscoelastic moduli with strain is not universal and depends on the mesoscopic structure and how it is structured at high deformations in the material [4]. Contrary to the SAOS protocol, as the shear strain increases, the sinusoidal response to shear stress is distorted (see Fig. 3.2). In this case, the experiment is referred to as LAOS protocol (Large Amplitude Oscillatory Shear).

Figure 3.2. Schematic representation of linear and nonlinear viscoelasticity regions for sinusoidal shear strain applied. The distorted stress (upper-right curve) corresponds to $\sigma/\sigma_0 = \sin(t) + 0.1 \sin(3t)$ at a fixed angular frequency ($\omega = 1 s^{-1}$) where σ_0 correspond to the maximum shear stress. Image modified from [4].

A number of different analysis frameworks have been used to characterize LAOS deformation. These mainly fall into three categories: 1) methods that extend the liner regimen Fourier analysis to higher harmonics [76–78], 2) methods that geometrically decompose the LAOS response into contributions depending on strain and strain rate [79–81], and derivate-based methods which use the trajectory of the response to define time-resolved moduli [14,82,83]. The stress is typically independent of the strain direction for the first category. Under this hypothesis, the shear stress is considered an odd function concerning the shear direction, which mathematically can be written as $\sigma[-\gamma(t), -\dot{\gamma}(t)] = -\sigma[\gamma(t), \dot{\gamma}(t)]$. Under this symmetry consideration, it is possible to calculate the nonlinear stress as a combination of linear shear stress characterized by different amplitudes and shift phases, *i.e.*,

$$\sigma(t) = \sum_{n=1,odd} \sigma_n \sin(n\omega t + \delta_n), \qquad (3.10)$$

where the amplitudes σ_n and shift phases δ_n depends of the shear strain and the frequency [4,76–78]. The form and how the shear stress change due of higher contribution can be visually quantified trough Lissajous-Botwich (LB) curves.

The LB curves are closed curves for an intracycle shear strain applied: 1) 3D LB curves $(\sigma/\sigma_0 vs. \gamma/\gamma_0 \text{ and } \dot{\gamma}_0/\dot{\gamma}_0)$, 2) elastic representation (projection in the plane $\sigma/\sigma_0 vs. \gamma/\gamma_0$), and 3) viscous representation (projection in the plane $\sigma/\sigma_0 vs. \dot{\gamma}_0/\dot{\gamma}_0$. Here, σ_0 , γ_0 , and $\dot{\gamma}_0$ are the maximum shear stress, maximum shear strain, and maximum shear rate in an oscillation cycle, respectively, and thus scale the axes. In the elastic representation in the LVR, if there is no shift phase between shear stress and shear strain, the loci is a line (called solid-like behavior); if the shift phase is equal to $\pi/2$, the loci is a circle (called fluid-like behavior), and if the shift phase is $\pi/4 < \delta < \pi/2$ the loci is ellipsoidal which correspond to viscoelastic behavior. Fig. 3.3 shows the construction of LB curves from the shear stress response for elastic, viscous, and viscoelastic behavior. In the viscous representation, the shape of the LB curves changes; for a solid-like behavior, the loci change to a circle; for fluid-like behavior, the loci change to a line; and for viscoelastic behavior, the loci remain unchanged. The change of loci curves between both representations is due to the shift phase of the response ($\sigma/\sigma_0 = sin(\omega t + \delta)$) is relative to elastic representation ($\gamma/\gamma_0 = sin(\omega t)$: $\delta = 0$ for solid-like behavior, $\delta = \pi/2$ for fluids-like behavior), and viscous representation ($\dot{\gamma}_0/\dot{\gamma}_0 = \cos(\omega t)$ where $\dot{\gamma}_0 = \omega \gamma_0$: $\delta = \pi/2$ for solid-like behavior, $\delta = 0$ for fluids-behavior).

Figure 3.3. Viscoelasticity response in the LVR region: a) Normalized stress as a function of the time for different rheological responses, and b) Lissajous-Botwich curves construction for different rheological responses in the elastic representation. The shear strain applied is a sinusoidal function.

In equation 3.10, the contributions for harmonics higher than third harmonic decay could be rapidly and can be neglected in the expansion for n > 5, 7, 9, In the work of Neidhöfer *et al*. [84], they supposed the contribution of the 3rd harmonic equal to 10 %, which can follow that:

$$\sigma(t)/\sigma_1 = \sin(\omega t + \delta_1) + 0.1\sin(3\omega t + \delta_3).$$
 (3.11)

In the upper-right curve of Fig. 3.2 shows the sinusoidal shear stress distorted (rectangular-like shape) for $\delta_3 = 0$; here, sinusoidal shear stress is distorder for $\delta_1 = 0$ (solid-like behavior). In the Fig. 3.4a can be see the LB curves for $\delta_3 = 0$ for three different rheological behavior, *i. e.*, how are the distorted LB curves in the elastic representation from the linear rheological response (Fig. 3.3b shows the LB curves for linear response). Fig. 3b – Fig. 3d shows the LB curves for different values δ_3 which is the results of the deformation of sinusoidal shear stress: if $\delta_3 = 90^{\circ}$, the shear stress signal distorted to a backward tilted shoulder, if $\delta_3 = 180^{\circ}$, the shear stress distorted to a triangular-like shape, and if $\delta_3 = 270^{\circ}$, the shear stress signal distorted to foreword tilted shoulder [4]. In summary, the distortion in the linear shear stress can be visualized as distortion in the LB curves for the linear response.

The harmonic-based techniques do capture all of the non-linearity of the LAOS data, but aside from the third harmonic, no specific meaning has been successfully assigned to each of these values, making comparisons challenging at large amplitudes [85]; for example, to describe a butterfly-like shape of LB curves [86]. Also, the molecular origin of the distortion of shear stress response for high strain values is unclear. Maybe the most simplistic model is the one proposed by Hyun *et al.* [87]; they investigated a possible mechanism of LAOS behavior using a network model composed of segments and junctions. A segment is part of a macromolecular chain, and junctions are where intermolecular interactions are located. Segments are lost and created during flow, and their dynamics can be characterized by a creation rate f(t) and a loss rate g(t). This approximation is too simplistic; however, it qualitatively predicts various behaviors observed in LAOS data and thus rationalizes the viscoelastic modulus responses in terms of the model parameters.

Figure 3.4. Normalized LB curves in the elastic representation at different third phase angles: a) $\delta_3 = 0^\circ$, b) $\delta_3 = 90^\circ$, c) $\delta_3 = 180^\circ$, and d) $\delta_3 = 270^\circ$ at a fixed angular frequency ($\omega = 1 \ s^{-1}$). The first shift phase corresponds to three different rheological behaviors: $\delta_1 = 0^\circ$ (elastic), $\delta_1 = 45^\circ$ (viscoelastic), and $\delta_1 = 90^\circ$ (viscous). Image from [4].

LAOS experiments can explore the full range between steady-state and strongly timedependent behaviors by investigating the effect of two characteristic dynamic variables, the Deborah number, *De*, and the Weissenberg number, *Wi*. Deborah number, *De* = τ_R/T , is defined as the ratio of a characteristic time scale of a material, τ_R , and a characteristic time scale of observation of the deformation process, *Td*, which is the inverse of angular frequency *T* = $1/\omega$ in oscillatory flow. The latter, *Wi* = τ_R/T_d , characterizes the ratio of elastic to viscous forces, *i.e.*, the ratio of τ_R and a characteristic time of the deformation process *Td*. For steady shear, *Td* is the inverse of the instantaneous shear rate $T_d = 1 / \dot{\gamma}$, whereas for oscillatory shear, $T_d = 1 / \dot{\gamma}_0 =$ $1 / (\omega \gamma_0)$ [4]. An appealing feature of LAOS in Maxwellian fluids is that the harshness of the flow's time dependence, relative to the fluid's intrinsic relaxation timescale, can be tuned by varying ω in the applied oscillation without sudden jumps in the strain input, as in step experiments [13].

LB curve for Maxwellian fluids in the LVR can be tuned by imposing ω and γ_0 where both quantities have a relationship with Deborah and Weissenberg numbers: $De = \tau_R \omega$, and $Wi_0 = \tau_R (\omega \gamma_0)$ is the amplitude Weissenberg number. For viscoelastic moduli form Maxwellian fluids (Eq. 3.5 and 3.6), G' and G'' can be written in terms of *De* as:

$$G' = G_0 \frac{De^2}{1 + De^2},$$
(3.12)

$$G'' = G_0 \frac{De}{1 + De^2}.$$
 (3.13)

On the other hand, manipulating the expression for shear stress in terms of viscoelastic moduli $(\sigma = \gamma_0 [G' \sin(\omega t) + G'' \cos(\omega t)]),$ has the following:

$$[\sigma - \gamma_0 G' \sin(\omega t)]^2 = [\gamma_0 G'' \cos(\omega t)]^2,$$

$$\sigma^2 - 2\sigma\gamma_0 G' \sin(\omega t) + \gamma_0^2 G'^2 \sin^2(\omega t) = \gamma_0^2 G''^2 [1 - \sin^2(\omega t)].$$

For a sinusoidal shear deformation $\gamma = \gamma_0 \sin(\omega t)$, and substituting in the last equation, it follows that:

$$\sigma^{2} - 2\sigma\gamma G' + \gamma^{2}(G'^{2} + G''^{2}) = \left(G''\frac{Wi_{0}}{De}\right)^{2}.$$
(3.14)

Eq. 3.14 allows us to describe the LB curves according to the previous mentions: for a solid-like behavior ($G' \neq 0$ and G'' = 0), a straight line, for a fluid-like behavior (G' = 0 and $G'' \neq 0$), a circle, and an ellipsoid for a viscoelastic behavior ($G' \neq 0$ and $G'' \neq 0$). For all cases, the maximum stress always occurs in the first quadrant (all positive variables). For Maxwellian fluids, the oscillatory shear strain is probed by selecting γ_0 . LAOS protocol is not limited to small values, producing deviations from linear behavior, *e.g.*, the maximum shear strain deviation from the first quadrant to the second quadrant.

For solid-like materials, *e.g.*, gels, the nonlinear stress-strain curve is directly related to the recovery energy of the material and, in the background, to its structure and how it changes while it deforms until it breaks. To describe LAOS experiments, phenomenological network models that are based on a continuum approach are often used. Blatz, Sharda, and Tschoegl proposed a BST

model involving non-Hookean relation between deformation energy and deformation [88]. For an applied shear stress, the relationship to the shear stress is:

$$\sigma = \frac{2G_0}{n_{BST}} \frac{\lambda^{n_{BST}} - \lambda^{-n_{BST}}}{\lambda - \lambda^{-1}},\tag{3.15}$$

where $\lambda = \frac{1}{2}\gamma + (1 + \frac{1}{4}\gamma^2)^{1/2}$ (or $\gamma = \lambda - \lambda^{-1}$), G_0 is the elasticity modulus, and n_{BST} is a nonlinear fitting exponent. Interestingly, for $n_{BST} = 2$, the last equation to reduce the ideal rubber elasticity case is $\sigma = G\gamma$.

To understand the nonlinearity parameter n_{BST} through a molecular interpretation, a scaling model (based on the fractal structure of the polymers), a FENE model (based on the finite extensibility of the polymers), and a rod and coil model (based on the biochemical microstructure of gelatin) were developed by Groot *et al.* [89]. It was found that the scaling model, the fractal dimension d_f could better quantitatively describe the stress-strain curve. The nonlinear exponent can be related to the fractal dimension of the network and swelling exponent as:

$$n_{BST} \approx \frac{d_f}{d_f - 1} = \frac{1}{1 - \nu}.$$
 (3.16)

Throughout the text, the equations 3.15 and 3.16 refer to the BST scaling model

3.2 Rheometry

Determining the rheological properties of any material when subjected to flow involves measuring stress and strain. A rheometer is an instrument that can determine these quantities from the torque, angular velocity, and characteristic dimensions of the geometries used. The coneplate geometry was used in this work, and in the following paragraphs, how to determine stress and shear rate will be discussed. The discussion is based on the textbook Rheology principles: *Measurements and Applications* by Macosko, C. W. [90].

The conservation equations of mass, momentum, and energy describe the motion of any material body. For their derivation, a control volume element dV is used. In the mass balance, a flow of matter through the control volume is considered, where the rate of mass change ρdV will be equal to the mass flow through its surface, mathematically expressed as:

$$\frac{d}{dt} \int_{V} dV \rho = -\int_{S} dS(\,\widehat{\boldsymbol{n}}\rho \cdot \mathbf{v}). \tag{3.17}$$

Using a divergence theorem over the right side (Ec. 3.17), the surface integral can be written in terms of integral over the volume. Also, the time derivate can be included in the integral, and since it is an arbitrary volume, a differential expression can be obtained as:

$$\int_{V} dV \left(\frac{\partial \rho}{\partial t} + \nabla \cdot \rho \mathbf{v} \right) = 0 \implies \frac{\partial \rho}{\partial t} = -\nabla \cdot \rho \mathbf{v}.$$
(3.18)

The last equation is called the continuity equation. We can write Eq. 3.18 in terms of the material derivative, $D/Dt = \partial/\partial t + \nabla \cdot \mathbf{v}$, which describes the time rate of change of some physical quantity of a material element embedded into a macroscopic velocity field. Eq. 3.18 in terms of material derivative is:

$$\frac{D\rho}{Dt} = -\rho \nabla \cdot \mathbf{v}.$$
(3.19)

Thus, the continuity equation is in the Lagrangian representation, and it can be seen that if the fluid is incompressible ($\rho = cte$), the velocity divergence is zero.

Performing the momentum balance, the change of momentum in the volume is equal to three contributions: the first is determined by the momentum flux on the surface $-(\mathbf{\hat{n}} \cdot \mathbf{v})dS$ per unit volume $\rho \mathbf{v}$, the second is the effect of the stresses acting on the surface $\sigma_n dS = \mathbf{\hat{n}} \cdot \overline{\sigma} dS$ (σ_n denote the stress over the surface, and $\overline{\sigma}$ the stress as a tensor quantity), and the third corresponds to the change of momentum due to the gravitational field $\rho \mathbf{g} dV$. In mathematical terms, the last balance is:

$$\frac{d}{dt} \int_{V} dV \rho \mathbf{v} = -\int_{S} dS(\hat{\mathbf{n}} \cdot \mathbf{v}) \rho \mathbf{v} + \int_{S} dS(\hat{\mathbf{n}} \cdot \overline{\overline{\sigma}}) + \int_{V} dV \rho \mathbf{g}.$$
(3.20)

Using the divergent theorem and including the time derivative in the integral, the differential equation for momentum balance is:

$$\int_{V} \left(\frac{\partial \rho}{\partial t} + \nabla \cdot \rho \mathbf{v} \mathbf{v} - \nabla \cdot \overline{\overline{\sigma}} - \rho \mathbf{g} \right) = 0 \implies \frac{\partial \rho}{\partial t} = -\nabla \cdot \rho \mathbf{v} \mathbf{v} + \nabla \cdot \overline{\overline{\sigma}} + \rho \mathbf{g}.$$
(3.21)

In terms of material derivative, the Eq. 3.21 can be written as:

$$\rho \frac{D\mathbf{v}}{Dt} = \nabla \cdot \overline{\overline{\boldsymbol{\sigma}}} + \rho \boldsymbol{g}. \tag{3.22}$$

Eqs. 3.19 and 3.22 are the expression that describes the motion of any material body, and their components depend on the coordinate system in which it works.

On the other hand, to know the flow's change of velocities, we consider two points immersed in the medium, P, and Q, separated by a distance dx. If the velocity within the fluid depends on position and time, the velocities differences between P and Q is:

$$d\mathbf{v} = \frac{\partial \mathbf{v}}{\partial \mathbf{x}} \cdot d\mathbf{x} = \overline{\overline{L}} \cdot d\mathbf{x},$$
(3.23)

where \overline{L} is a gradient tensor of velocities, which has two directions: 1) of the velocity, and 2) of its gradient. In terms of its components, the tensor has the form:

$$\nabla \mathbf{v} = \overline{\mathbf{L}}^{\mathrm{T}} = \sum_{i} \sum_{j} \widehat{\mathbf{x}}_{i} \widehat{\mathbf{x}}_{j} \frac{\partial \mathbf{v}_{j}}{\partial \mathbf{x}_{i}}.$$
(3.24)

Equation 3.24 allows us to calculate the value of \overline{L} , explicitly. Additionally, the velocity tensor can be decomposed into its symmetric, \overline{S} , and antisymmetric, \overline{A} , parts as follows:

$$\overline{\overline{L}} = \frac{1}{2} \left(\overline{\overline{L}} + \overline{\overline{L}}^T \right) + \frac{1}{2} \left(\overline{\overline{L}} - \overline{\overline{L}}^T \right) = \overline{\overline{S}} + \overline{\overline{A}},$$
(3.25)

where $2\overline{S}$ represents the strain rate and $2\overline{A}$ the vorticity. In this way, we can know the velocity profile when, for example, when shear strain is applied.

The Cone - Plate geometry is shown in Fig. 3.5; the geometry consists of a plate of radius R, a truncated cone of the same radius which forming an angle β concerning the plate. The cone rotates with an angular velocity Ω , and the rod supporting the geometry measures torque M. Spherical coordinates are convenient for finding the associated shear stress and shear rate. It is important to consider that β is small enough to neglect inertial effects. By symmetry, $v_{\theta} = v_r = 0$, and there is only dependence on $v_{\phi}(\theta)$. In spherical coordinates, \overline{L} is:

$$\overline{\overline{L}} = \begin{bmatrix} 0 & 0 & -\frac{\mathbf{v}_{\phi}}{r} \\ 0 & 0 & -\frac{\mathbf{v}_{\phi}}{r} \cot(\theta) \\ 0 & \frac{1}{r} \frac{\partial \mathbf{v}_{\phi}}{\partial \theta} & 0 \end{bmatrix}.$$
 (3.26)

Figure 3.5 Characteristic lengths of the Cone - Plate geometry where the angle β is exaggerated. Image from [90].

Calculating the strain rate with the definition of equation 3.24, and assuming a small angle $(\beta < 0.10 \ rad \Rightarrow \cot(\pi/2 - 0.1) < 0.1)$, the relationship for $2\overline{\overline{S}}$ can be approximated as follows:

$$2S_{\phi\theta} = \frac{1}{r} \frac{\partial \mathbf{v}_{\phi}}{\partial \theta} - \frac{\mathbf{v}_{\phi}}{r} \cot(\theta) \approx \frac{1}{r} \frac{\Delta \mathbf{v}_{\phi}}{\Delta \theta} \approx \frac{1}{r} \frac{\mathbf{v}_{\phi} \left(\frac{\phi}{2} - \beta\right) - \mathbf{v}_{\phi} \left(\frac{\pi}{2}\right)}{\beta}.$$
(3.27)

Of the no-slip condition, must be met that $v_{\phi}(\pi/2) = 0$, and $v_{\phi}(\pi/1 - \beta) = \Omega r \sin(\pi/2 - \beta) \simeq \Omega r$, resulting that Eq. 3.27 change to:

$$\dot{\gamma} = 2D_{\phi\theta} \approx \frac{1}{r} \frac{\Omega r - 0}{\beta} \approx \frac{\Omega}{r}$$
(3.28)

Now, for to obtain a stress expression, taking the equation of motion (Eq. 3.22) in spherical coordinates, and in the absence of a gravitational field, we follow that:

$$\frac{1}{r}\frac{\partial\sigma_{\phi\theta}}{\partial\theta} + \frac{2}{r}\cot(\theta)\,\sigma_{\phi\theta} = 0.$$
(3.29)

Integrating the last equation:

$$\sigma_{\phi\theta} = \frac{cte}{\sin^2(\theta)} \approx cte'. \tag{3.30}$$

For a value of $\beta < 0.10 \, rad$, the $\sin^2(\pi/2 - \beta)$ is bounded between 0.99 and 1, so that the value of $\sigma_{\phi\theta}$ can be considered constant. From the balance of the torque in the geometry, we finally have an expression for the stress:

$$M = \int_0^{2\pi} \int_0^R dr d\theta r^2 \,\sigma_{\phi\theta} \implies \sigma_{\phi\theta} = \frac{3M}{2\pi R^3}$$
(3.31)

Equations 3.28 and 3.31 allow for the calculation of the shear strain and shear stress, respectively, with the rheometer's torque and angular velocity measurement.

3.2 Diffusion wave spectroscopy and microrheology

An alternative to mechanical rheology (sometimes called macro rheology) is the microrheology technique. In microrheology, the principal characteristic is the size of the probe test; here, the length of the probe test is in micrometers, while in macro rheology, the length of the probe test is the rheological geometry size. Diffusion wave spectroscopy (DWS) is a passive microrheological technique that essentially measures the mean square displacement (MSD) of the Brownian motion of colloidal particles embedded in the material. The central premise for obtaining the medium's viscoelastic properties is that the particles' Brownian motion is affected

by the structure of the medium. This way of obtaining the viscoelasticity of materials was proposed by Mason & Weitz in 1995 [91], whose technique was first reported in the early 1990s [92,93]. Since then, DWS has greatly improved the ability to display the viscoelasticity of complex fluids, concerning that obtained by mechanical rheology, by the ability to access shorter time scales $t\sim 10^{-6} - 10^2 s$ corresponding to higher frequencies $\omega \sim 10^{-2} - 10^6 s^{-1}$.

The first works on micro-rheology in complex fluids by DWS were in the early 1990's by Mason *et al.* [94] and Mason & Weitz [91] for colloidal hard sphere solutions. In the end of 90's Xu, Palmer & Weitz [95] reported for the first time the micro-rheology of F-actin filament solutions as a model of semiflexible polymers, finding the dependence of the complex modulus $|G^*(\omega)| \propto \omega^{0.78\pm0.10}$, according to the Morse model, which predicts a dependence on $G^*(\omega) \propto (i\omega)^{3/4}$ [96]. Since then, several works on micro-rheology in living polymers and biopolymers have been published, and nowadays, DWS remains a technique of special interest for the study of polymeric dissolutions, given the information that can be obtained. For example, in 2017, Krajina *et al.* [97] revealed the different modes of relaxation of DNA chains. In 2018, Gartin & Stradner [98] studied the interaction of tracer particles with protein in concentrated dissolutions, validating the micro-rheology technique in this concentration regime. The following sections discuss DWS theory as a method for determining the MSD of particles embedded in the material and micro-rheology as to how viscoelasticity properties can be extracted from the MSD of these particles.

3.2.1 Diffusion Wave Spectroscopy

Theory of DWS. A light scattering experiment consists of an incident of a laser light beam (characterized by a wavevector k_0) on a sample. The scattered light due to the dielectric constant difference between the particles and the solvent is collected in a detector placed at an angle θ in the direction of the wave vector k_s . The scattered electric field depends on the position of the particles embedded in the fluid, which vary in time due to motion under thermal interactions. Fluctuations in the motion of the particles imply that the scattered light fluctuates in time and implicitly provides information on the structure and dynamics of the system [99]. If the particle concentration is sufficiently dilute, only a single scattering event will occur, and this experiment is known as Dynamic Light Scattering (DLS) [99]; if the particle concentration is high, it is a multiple light scattering experiment. The following discussion about the DWS theory is based on the text

Diffusing-Wave Spectroscopy in Dynamic Light Scattering: The Method and Some Applications by Weitz D. A., Pine D. J. (1993), edited by W. Brown [23].

In DLS, the electromagnetic wave induces a dipole moment that radiates in all directions. If the field scattered by a particle is E_0 , the total field E is equal to the superposition of the fields scattered by N particles:

$$E(t) = \sum_{i=1}^{N} E_0 \exp[i\boldsymbol{q} \cdot \boldsymbol{r}_i(t)], \qquad (3.32)$$

where $q = k_s - k_0$ is the scattering wavevector whose magnitude is $q = \frac{4\pi n}{\lambda} \sin(\frac{\theta}{2})$, and $r_i(t)$ is the position of the ith-particle. The exponential function represents the phase shift introduced by the differences in the optical path each wave will take after the scattering event. This change will introduce an interference pattern in the detection plane known as a speckle pattern, which is related to fluctuations in the light intensity, *I*, directly related to the motion of the particles.

The temporal autocorrelation function can describe the fluctuations in the light scattering:

$$g_{(2)}(t) = 1 + \beta |g_{(1)}(t)|^2, \qquad (3.33)$$

where $g_{(2)}$ is a correlation function over the light intensity, $g_{(1)}$ is the correlation function over the electric field, and β is a constantar determined primily by the collection optics of the experiment. Equation 3.33 is a relationship between $g_{(1)}$ and $g_{(2)}$ knowing as the Siegert relation. In general, $g_{(1)}$ is given by:

$$g_{(2)}(t) = \frac{\sum_{i=1}^{N} \sum_{j=1}^{N} \langle \exp\{i \boldsymbol{q} \cdot [\boldsymbol{r}_{i}(0) - \boldsymbol{r}_{j}(t)]\}\rangle}{\sum_{i=1}^{N} \sum_{j=1}^{N} \langle \exp\{i \boldsymbol{q} \cdot [\boldsymbol{r}_{i}(0) - \boldsymbol{r}_{j}(0)]\}\rangle}.$$
(3.34)

For the case of uncorrelated and non-interacting particles, the cross-terms vanish and $g_{(1)}$ changes to $g_{(1)} = \langle \exp[-i\mathbf{q} \cdot \Delta \mathbf{r}(t)] \rangle$ (here, $\Delta \mathbf{r}(t) \equiv \mathbf{r}(t) - \mathbf{r}(0)$). Now, if $\Delta \mathbf{r}(t)$ is a Gaussian variable the last equation can be written as:

$$g_{(1)}(t) = \exp\left[-q^2 \frac{\langle \Delta r^2(t) \rangle}{6}\right].$$
 (3.35)

DWS is an extension of DLS where multiple scattering events occur. In multiple light scattering, two scales characterize the scattering and transport of light: 1) The mean free path l between two scattering events, and 2) the mean free path of transport, which is the distance where light propagation is randomized. The above quantities are given by:

$$l = \frac{1}{\rho a} \text{ for dilute suspensions,}$$
(3.36)

$$l^* = \frac{l}{\langle 1 - \cos\theta \rangle}.$$
(3.37)

Here, ρ is the number density of the particles, and a is the total scattering cross section for a single particle. Two things must be considered to obtain the autocorrelation function: i) Approximating the scattering light intensity along each photon path through the medium, and ii) averaging the scattering events instead of considering individual events.

First, we consider a DWS measurement in a transmission experiment; light from a laser is incident on one side of a planar sample of thickness with $L \gg l^*$, and scattered light is collected from a small area on the opposite side (see Fig. 3.6). A simple photon passing through the sample undergoes *n* scattering events and emerges with a phase that depends on its total path length *s* such as shown in Fig. 3.6. The total path length for a single photon scattering *N* times is:

$$s = \sum_{i=0}^{N} |\mathbf{r}_{i+1} - \mathbf{r}_{i}| = \sum_{i=0}^{N} \left(\frac{\mathbf{k}_{i}}{|\mathbf{k}_{i}|}\right) \cdot (\mathbf{r}_{i+1} - \mathbf{r}_{i}),$$
(3.38)

where \mathbf{k}_i is the wavevectors of the light after *i* scattering events, \mathbf{r}_i is the position of particle *i* for $i \ll 1 \ll n$, \mathbf{r}_0 is the position of the laser, and \mathbf{r}_{N+1} is the position of the photo multiple detector (PMT). Because the scattering process is quasi-elastic, all wavevectors have the same magnitude, $|\mathbf{k}_i| = k_0$. The total phase shift $\phi(t)$ of the photon after passing from the laser to the detector is $\phi(t) = k_0 s(t) = \sum_{i=0}^{N} \mathbf{k}_i \cdot [\mathbf{r}_{i+1}(t) - \mathbf{r}_i(t)]$. So, the total field and the detector is the superposition of the fields from all light paths through the sample to the detector, and can be written as:

$$E(t) = \sum_{p} E_{p} e^{i\phi_{p}(t)},$$
(3.39)

here, Σ_p represents the sum over paths, and E_p is the amplitude of the field from path p at the detector. Is interesting to note that Eq. 3.39 has the same form as its single-scattering counterpart (see Eq. 3.32). Equation 3.39 involves two sums, one over the light path and another over the particle in each path. E_p depends on the number of scattering events in a given path and the laser beam intensity; the determination of E_p adds some complexity to the multiple-scattering theory.

Figure 3.6. The upper-right hand shows a single path through a sample of multiple light-scattering events, and the lower-left hand shows a transmission geometry. In transmission geometry, the light incident at a point r_{in} (laser) on the face of a slab of thickness L is collected from a point r_{out} (PMT) on the opposite face of a slab.

The field autocorrelation function is obtained from Eq. 3.39, which is:

$$g_{(1)}(t) = \left(\frac{\langle E(0)E^*(t)\rangle}{\langle |E|^2\rangle}\right) = \frac{1}{\langle I\rangle} \left\langle \left(\sum_p E_p e^{i\phi_p(0)}\right) \left(\sum_p E_p^* e^{-i\phi_p(t)}\right)\right\rangle,\tag{3.40}$$

where $\langle I \rangle$ is the total average scattered intensity at the detector. For independent particles, the fields from different paths are, to a very good approximation, uncorrelated. In the last equation, the terms with $p \neq p'$ do not contribute, and we obtained that:

$$g_{(1)}(t) = \langle \sum_{p} \frac{\left|E_{p}\right|^{2}}{\langle I \rangle} e^{i[\phi_{p}(0) - \phi_{p}(t)]} \rangle = \sum_{p} \frac{\langle I_{p} \rangle}{\langle I \rangle} \langle e^{i[\phi_{p}(0) - \phi_{p}(t)]} \rangle, \tag{3.41}$$

where $\langle I_p \rangle \equiv \langle |E_p|^2 \rangle$ is the average intensity from path *p* and the independence of the phase and field amplitude E_p at the detector is assumed.

The next step is obtaining an expression for the change in the phase of the scattered light resulting from particle motion. Is possible to obtain and expression for the phase which is:

$$\Delta \phi_p(t) = \sum_{i=1}^{N} \boldsymbol{q}_i \cdot \Delta \boldsymbol{r}_i(t), \qquad (3.42)$$

where the magnitude of q is $q = 2k_0 \sin(\frac{\theta}{2})$. Taking the average over square of phase shift in the Eq. 3.42,

$$\langle \Delta \phi_p^2(t) \rangle = \sum_{i=1}^N \sum_{j=1}^N \langle [\boldsymbol{q}_i \cdot \Delta \boldsymbol{r}_i(t)] [\boldsymbol{q}_j \cdot \Delta \boldsymbol{r}_j(t)] \rangle$$

$$= \sum_{j=1}^N \langle [\boldsymbol{q}_i \cdot \Delta \boldsymbol{r}_i(t)]^2 \rangle = \frac{1}{3} N \langle \boldsymbol{q}^2 \rangle \langle \Delta \boldsymbol{r}^2(t) \rangle.$$

$$(3.43)$$

Here, we have assumed the independence of successive phase factors $q_i \cdot \Delta r_i(t)$, and that the scattering and displacement vectors are independent. Also, the average over q^2 is weighted by the single-particle formfactor and can be expressed in terms of the mean free paths, *i.e.*, $\langle q^2 \rangle = \langle \left[2k_0 \sin\left(\frac{\theta}{2}\right)\right]^2 \rangle = 2k_0^2 \langle 1 - \cos(\theta) \rangle = 2k_0^2 \frac{l}{l^*}$. Insert the last equation in Eq. 3.43 and considering that for $N \gg 1$ the total path lengt trough the sample is given by s = N/*, can be follow that:

$$\langle \Delta \phi_p^2(t) \rangle = \frac{1}{3} \frac{s}{l} 2k_0^2 \frac{l}{l^*} \langle \Delta r^2(t) \rangle = \frac{2}{3} k_0^2 \langle \Delta r^2(t) \rangle \frac{s}{l^*}.$$
(3.44)

Return to Eq. 3.41, if replaced the fraction of scattered in path p, $\langle I_p \rangle / \langle I \rangle$, with the fraction of the scattered intensity in paths of lengths *s*, *P*(*s*), and using the equation 3.44, it can be seen that:

$$g_{(1)}(t) = \sum_{s} P(s) \exp\left(-\frac{1}{3}k_0^2 \langle \Delta r^2(t) \rangle \frac{s}{l^*}\right).$$
(3.45)

In the last equation, we assume that, $\Delta \phi_p(t)$ is a random Gaussian variable, and according to the central limit theorem, $\langle e^{-i\Delta \phi_p(t)} \rangle = e^{-\frac{\langle \Delta \phi_p^2(t) \rangle}{2}}$. Eq. 3.45 can be written in terms of diffusion coefficient, D. For example, for diffusing colloid particles $\langle \Delta r^2(t) \rangle = 6Dt$ and the Eq. 3.45 is written as $g_{(1)}(t) = \sum_s P(s) \exp\left(-2k_0^2 Dt \frac{s}{l^*}\right)$. Thus, the calculation of the autocorrelation function is reduced to determine the path-length distribution function P(s) through a sample. This calculation is greatly facilitated by passing to the continuum limit:

$$g_{(1)}(t) = \int_0^\infty ds P(s) \exp\left(-\frac{1}{3}k_0^2 \langle \Delta r^2(t) \rangle \frac{s}{l^*}\right).$$
(3.46)

To calculate P(s), consider a simple thought experiment: An instantaneous pulse of light is incident over some sample area; light entering the sample is multiplied and scattered, and a random walk is performed until it scapes. Photons arriving at time t after the incident pulse will have traveled a distance s = ct through the sample, where c is the average speed of light within the medium. The flux $J_{out}(r_{out}, t)$ of photons arriving at the point r_{out} will be directly proportional to the fraction of photons that travel a distance s = ct, that is, to P(s). For length scales greater than the transport mean free path, the transport of light is accurately described by the diffusion equation:

$$\frac{\partial U}{\partial t} = D_l \nabla^2 U \tag{3.47}$$

where U is the energy density of light in the sample, and $D_l = cl^*/3$ is the diffusion coefficient of light. To calculate P(s), we consider an instantaneous light pulse propagating along the z-direction, which is incident at z = 0 on a planar sample of thickness L. Since the light is randomized within the sample over a distance comparable with l^* , we describe the incident pulse as an instantaneous source of diffusing light, a distance $z_0 \sim l^*$ inside the sample. Thus, the initial condition is:

$$U(z,t=0) = U_0 \delta(z - z_0, t).$$
(3.48)

In addition to this initial condition, we must specify the diffusing light's boundary conditions. These are obtained by requiring that for t > 0, the net flux of diffusing light into the sample is zero [92]. The result is the boundary condition is:

$$U + \frac{2}{3}l^*\hat{\boldsymbol{n}} \cdot \nabla U = 0 \tag{3.49}$$

where \hat{n} is the unit normal vector directed out of the sample. Using the initial condition given in Eq. 3.48 and the boundary condition given in Eq. 3.49, we can solve the diffusion equation (see Eq. 3.47) for any particular experimental geometry and obtain a solution for U(r) valid for all points within the sample. From this solution, we can calculate the time-dependent flux of light emerging from the sample at the exit point r_{out} , using Fick's law. Since all light emerging from the sample a distance s = ct, the fraction of light, P(s), that travels a distance s trough the same is simply proportional to the flux emerging at time t. So, P(s) is given by

$$P(s) \propto |\boldsymbol{J}_{out}(\boldsymbol{r}, t)|_{\boldsymbol{r}_{out}} = D_l |\boldsymbol{\hat{n}} \cdot \nabla U|_{\boldsymbol{r}_{out}} = \left(\frac{cU}{2}\right)_{\boldsymbol{r}_{out}}.$$
(3.50)

On the other hand, the Laplace transform of diffusion equation (see Eq. 3.47) is:

$$\nabla^2 \widetilde{U} - \frac{3p}{l^*} \widetilde{U} = -\frac{3p}{l^*} U_{00}(\mathbf{r})$$
(3.51)

where, $\widetilde{U} \equiv \widetilde{U}(\mathbf{r},p)$ is the Laplace transform of $U(\mathbf{r},s)$, $\widetilde{U}(\mathbf{r},p) \equiv \int_0^\infty ds e^{-ps} U(\mathbf{r},s)$, and $U_{00}(\mathbf{r}) = \lim_{t \to 0} U_0 \delta(z - z_0, t)$. In solving this equation, we must also use the Laplace transform of the boundary conditions (see Eq. 3.49), *i.e.*, $\widetilde{U} + \frac{2}{3}l^*\widehat{\mathbf{n}} \cdot \nabla \widetilde{U} = 0$. These equations can then be solved using Green's function techniques to obtain $\tilde{U}(\mathbf{r}, p)$, as discussed by Carslaw & Jaeger [100]. Taking the Laplace transform of Eq. 3.50, we can relate this solution, $\tilde{U}(\mathbf{r}, p)$. So, the Laplace transform of P(s) is:

$$\tilde{P}(p) \equiv \int_0^\infty ds P(s) e^{-ps} \propto \left(\frac{cU}{2}\right)_{r_{out}}$$
(3.52)

For another hand, remember the equation for $g_{(1)}(t)$ (see Eq. 3.46) is it possible to see that

$$g_{(1)}(t) = \int_0^\infty ds P(s) e^{-ps} = \tilde{P}(p),$$
(3.53)

where, $p = \frac{1}{3}k_0^2 \frac{(\Delta r^2(t))}{l^*}$. From equations 3.52 and 3.53 can be followed that:

$$g_{(1)}(t) = \frac{\widetilde{U}(\boldsymbol{r}, \boldsymbol{p})_{\boldsymbol{r}_{out}}}{\widetilde{U}(\boldsymbol{r}, \boldsymbol{0})_{\boldsymbol{r}_{out}}},$$
(3.54)

Here, $\widetilde{U}(p)$ has been normalized so that $g_{(1)}(0) = 1$.

The most used geometry in the laboratory is the transmission one, which is helpful for probing motion over length scales much shorter than λ . For the case of a sample with parallel plane faces and with uniform illumination of a plane-wave beam, the autocorrelation function can be written as a function of the MSD (sometimes written as $\langle \Delta r^2(t) \rangle$). From the solution of the diffusion light equation $g_{(1)}$ is:

$$g_{(1)}(t) = \frac{\frac{L}{l^*} + \frac{4}{3}}{\left(1 + \frac{4}{9}x^2\right) \operatorname{senh}\left(\frac{L}{l^*}x\right) + \frac{4}{3}\operatorname{xcosh}\left(\alpha^*x\right)} \right]$$
(3.55)

with $x \equiv \sqrt{k_0^2 \langle \Delta r^2(t) \rangle}$ and $\alpha^* \equiv z_0/l^*$. The value $z_0 \sim l^*$, although numerical values are insensitive to the exact choice of z_0 , since $L \gg z_0$. A different choice of z_0 would affect only the first few steps in a random walk that consists of many steps. Backscattering geometry can also be used, as seen in Weitz & Pine's work [23]. One must guarantee that $9 \leq L/l^{\Lambda*} \leq 25$ [101,102], refers to the number of scattering events in the sample. If the relation L/l^* too small, diffusing light beams decreases; conversely, if L/l^* is large, the possibility for colloidal particle interactions among them increases [102,103].

Recently, a method was developed to circumvent absorption issues in DWS, deriving a correction to the theory [101]. They pointed out that absorption exponentially attenuates light paths according to their path length, cutting off the longest paths. Therefore, if P(s) is the path length distribution in the

absence of absorption, the path length distribution in the presence of absorption must be $P(s)e^{-s/l_a}$ as derived from first principles in [101]. Then, Eq. 3.53 can be rewritten as:

$$g_{(1)}(t) = \int_0^\infty ds P(s) \exp\left[\left(-\frac{1}{3}k_0^2 \langle \Delta r^2(t) \rangle \frac{l^*}{l_a}\right) \frac{s}{l^*}\right].$$
 (3.55)

The absorption effect represents a time delay in the time axis by l^*/l_a . As for the conventional DWS, a diffusion equation for the energy density is necessary to get an analytical expression for $g_{(1)}$. Therefore, an absorption term must be included to get the scattered photons' correct path distribution function. It is possible to derive a diffusion equation from the radiative transport equation with an absorption-independent diffusion coefficient [101]. The diffusion equation is written as:

$$\frac{\partial U}{\partial t} = D_l \nabla^2 U - \frac{c}{l_a} U, \qquad (3.56)$$

where is assuming that $l_a \gg l^*$ [103,104]. For the transmission geometry, following Ref. [101], the equation for the field autocorrelation function is:

$$g_{(1)}(t) = \frac{\left(1 + \frac{4}{9}\eta^2\right) \operatorname{senh}\left(\frac{L}{l^*}\eta\right) + \frac{4}{3}\eta \cosh\left(\frac{L}{l^*}\eta\right)}{\left(1 + \frac{4}{9}x_a^2\right) \operatorname{senh}\left(\alpha^* x_a\right) + \frac{2}{3}x_a \cosh(\alpha^* x_a)\right]} \begin{bmatrix} \operatorname{senh}(\alpha^* x_a) + \frac{2}{3}x_a \cosh(\alpha^* x_a) \end{bmatrix}$$
(3.57)
$$g_{(1)}(t) = \frac{\operatorname{senh}(\alpha^* \eta) + \frac{2}{3}\eta \cosh(\alpha^* \eta)}{\left(1 + \frac{4}{9}x_a^2\right) \operatorname{senh}\left(\frac{L}{l^*}x_a\right) + \frac{4}{3}x_a \cosh\left(\frac{L}{l^*}x_a\right)}$$

where $\eta \equiv \sqrt{3l^*/l_a}$ and $x_a \equiv \sqrt{k_0^2 < \Delta r^2(t) > +\eta^2}$. Interestingly, for $l_a \to \infty$, we recover the Eq. 3.55.

DWS experimental setup. The experimental setup was developed in the Fluids Complex Group at the Physics Institute at UNAM and was reported in a recent article review [105]. Fig. 3.7a. shows a diagram of the experimental setup; a brief description is provided below. A 532 *nm* laser beam (1) (Genesis MS SLM, USA) passes through a spatial filter (2), where it is expanded, and subsequently, a large pupil eliminates the external part of the Gaussian beam to approximate the beam to a plane wave (4). The beam shines the sample in a thermally stabilized bath (5) and (6). The scattered light passes through an achromatic doublet (7) and a crossed polarizer (8) to increase the autocorrelation response. Next, the light is collected by an optical fiber (OZoptics Inc) (9) and detected by two photomultipliers (Thorn EMI) (10). The autocorrelation function is obtained by a Digital correlation (Flex 02-08D/C, USA) in cross-correlation mode, controlled with a computer (11). For determining the values of *I** and *I*_a parameters necessary to perform the numerical

inversion of equations 3.55 and 3.57, a mirror (3) is set next to the spatial filter to make use of the branch framed by a red dashed square in Fig. 3.7b., where was used an integrating sphere (Oriel, Newport) (15). Between the mirror and the integrating sphere, set an optical filter and two diaphragms (13 and 14), separated at a fixed distance to obtain the input parameters for IAD: M_R (total reflectance) and M_T (total transmitance). The light signal is detected by a photomultiplier tube (Hamamatsu Photonics, Japan) (16) connected to a voltmeter (17). IAD method gives I^* , I_a , and the anisotropy factor g [106,107]. Fig. 3.7c. shows a photograph of the experimental setup.

Figure 3.7. (a) DWS experimental setup. The branch within the red dashed frame recovers the samples' optical parameters (l^* , l_a , and g). (b) Setup about the integrating sphere to obtain M_R (total reflectance), and M_T (total trnasmitation). (c) Experimental setup. Figures a) and b) modify from [105].

Inverse Adding Doubling (IAD) method. In this method, a general numerical solution for a radiative transport equation is given through the following steps: a) An educated guess for a set of the optical parameters is given. b) The samples' reflection and transmission are estimated using the adding doubling method. c) Transmittance and reflectance are compared with the experimental measurements. d) If the match is not good enough, the set of optical parameters is modified using a minimization algorithm [106,107]. The process is iteratively followed until a match with the experimental measurements is reached. The experimental and computational algorithms described in the literature where followed, and used the available open-source code. Definition of total reflectance M_R , and total transmittance M_T of light, on and through the sample, respectively, are:

$$M_R \equiv r_{std} \frac{R(r_s^{direct}, r_s) - R(0, 0)}{R(r_{std}, r_{std}) - R(0, 0)},$$
(3.58)

$$M_{R} \equiv \frac{T(r_{s}^{direct}, r_{s}) - T_{dark}}{T(0, 0) - T_{dark}}.$$
(3.59)

Each term in these equations is measured independently, and the way to measure them is according to Fig. 3.7b. and Fig. 3.7c. For total reflectance, r_{std} is the reflectance value of the integrating sphere walls, $R(r_{std}, r_{std})$ is the reflection measurement for a standard sample, $R(r_s^{direct}, r_s)$ is the reflection measurement for the sample, and R(0,0) is the measurement of light intensity without a sample when light is allowed to go out of the sphere. For total transmittance, T(0,0) is the measurement of light intensity when the light strikes inside the sphere without sample, $T(r_s^{direct}, r_s)$ is the transmission measurement through the sample, and T_{dark} is the measurement of the intensity of light with the entrance port blocked.

3.2.2 Miro-rheology

As mentioned above, the DWS technique provides the MSD of microspheres embedded in the material where the viscoelasticity properties of the medium influence the dynamics. The microspheres are subject to Brownian motion, which dynamic is described by the generalized Langevin equation:

$$m\frac{d\boldsymbol{\nu}}{dt} = \boldsymbol{f}_R(t) + \int_0^t dt' \zeta(t-t') \mathbf{v}(t'), \qquad (3.60)$$

where **v** is the velocity of the particle, and *m* is the mass of the particle. $f_R(t)$ represent the random forces acting on the particle (forces between particles and stochastic forces), and ζ is the hydrodynamic resistance as a memory kernel, and satisfying the fluctuation-dissipation theorem, $\langle f_R(t) \cdot f_R(t') \rangle = 3k_B T \zeta(t - t')$. From equation 3.60, Mason *et al.* [108] developed a generalized Stokes-Einstein equation. In the Laplace space, taking the Laplace transform of Eq. 3.60 and considering that $\zeta(t)$ is causal ($\zeta(t) = 0$ for $t < 0 \Rightarrow \int_0^t dt' \zeta(t - t') \mathbf{v}(t') = \int_0^\infty dt' \zeta(t - t') \mathbf{v}(t')$), can be follow that:

$$\tilde{\mathbf{v}}(s) = \frac{\tilde{f}(s) + m\mathbf{v}(0)}{\tilde{\zeta}(s) + sm}$$
(3.61)

where $\tilde{X}(s) \equiv \int_0^\infty dt X(t) e^{-st}$ denotes the Laplace transform. Multipliving by $\mathbf{v}(0)$ both sides of Eq. 3.61, and taking the average:

$$\langle \tilde{\mathbf{v}}(s)\mathbf{v}(0) \rangle = \frac{\langle \tilde{f}(s)\mathbf{v}(0) \rangle + m \langle \mathbf{v}(0)\mathbf{v}(0) \rangle}{\tilde{\zeta}(s) + sm}.$$
(3.62)

The term $\langle \tilde{f}(s)\mathbf{v}(0) \rangle = 0$ because both quantities are uncorrelated, and $m \langle \mathbf{v}(0)\mathbf{v}(0) \rangle = 3k_B T$ by equipartition energy theorem. So, the last equation is reduced to:

$$\tilde{\zeta}(s) + sm = \frac{3k_B T}{\langle \tilde{\mathbf{v}}(s)\mathbf{v}(0) \rangle}.$$
(3.63)

Using the relationship between the MSD and the velocities in the Laplace space, $\langle \Delta \tilde{r}^2(s) \rangle = \frac{2}{s^2} \langle \tilde{\mathbf{v}}(s) \mathbf{v}(0) \rangle$, the Eq. 3.63 is rewritten as:

$$\tilde{\zeta}(s) + sm = \frac{6k_BT}{s^2 \langle \Delta \tilde{r}^2(s) \rangle}.$$
(3.64)

The most important assumption is the Stokes relation; here, the hydrodynamic resistance takes the same form for all frequencies, and., *i.e.*,

$$\tilde{\zeta}(s) = 6\pi a \tilde{\eta}(s), \tag{3.65}$$

where $\tilde{\eta}(s)$ is the complex viscosity, and a is the de radius of the particle. This hypothesis considers that the complex fluid is treated as a continuum medium around the spherical particle; this argument is valid when the length scales of the structures giving rise to the elasticity are much smaller than the particle's radius. Finally, due to the relationship between the complex modulus and complex viscosity, $\tilde{G}(s) = s\tilde{\eta}(s)$, substituting the Eq. 3.64 into 3.63 can follow that:

$$\tilde{G}(s) = \frac{s}{6\pi a} \left[\frac{6k_B T}{s^2 \langle \Delta \tilde{r}^2(s) \rangle} - ms \right].$$
(3.65)

The first term of the right hand represents the thermal fluctuation-dissipation in the medium, and the second term represents the inertia of the colloid particle, which is only significant at high frequencies ($\omega > 10^6 s^{-1}$) for latex particles of micron size. To obtain the generalized Stokes-Einstein equation in the frequency domain using analytical continuation, *i.e.*, $s \rightarrow i\omega$. Neglecting the inertia term in Eq. 3.64 can be seen that:

$$\tilde{G}(s) = \frac{k_B T}{\pi a s \langle \Delta \widetilde{r}^2(s) \rangle} \xrightarrow{s \to i\omega} G^*(\omega) = \frac{k_B T}{\pi a(i\omega) \mathfrak{I}_u\{\langle \Delta \widetilde{r}^2(s) \rangle\}}.$$
(3.66)

Here, $\Im_u \{X(t)\} \equiv \int_0^\infty dt X(t) e^{-i\omega t}$ is the unilateral Fourier transform. The evaluation of Eq. 3.66 from MSD experimental data allows us to obtain the viscoelasticity properties of the medium where the

frequency of access is to the order of the fluctuation time of the particle. For example, for DWS measurements $10^{-6} s < t < 10 s$ are equivalent to $10^{-1} s^{-1} < \omega < 10^{6} s^{-1}$).

Different authors have followed several procedures to determine the unilateral Fourier transform. Mason estimate Eq. 3.66 expanding the MSD the locally around the frequency of interest using a power law and evaluating the logarithmic derivative [109]. The viscoelasticity moduli can be computed as $G'(\omega) = |G^*(\omega)| \cos(\pi \alpha(\omega)/2)$ and $G''(\omega) = |G^*(\omega)| \sin(\pi \alpha(\omega)/2)$, where the complex modulus is:

$$|G^*(\omega)| \approx \frac{k_B T}{\pi a \langle \Delta r^2 (1/\omega) \rangle \Gamma(1 + |\alpha(\omega)|)},$$
(3.67)

where $\alpha(\omega) \equiv \left(\frac{d\ln(\Delta r^2(t))}{d\ln t}\right)_{t=1/\omega}$ and Γ is the gamma function.

Another procedure is related to the relation between the dynamic relaxation modulus (see Eq. 3.9). Substituting the Eq. 3.9 into Eq. 3.66 in taking the inverse Laplace transform can be followed that:

$$\tilde{J}(s) = \frac{1}{s\tilde{G}(s)} \left(\frac{\pi a}{k_B T}\right) \langle \Delta \tilde{r}^2(s) \rangle \ or J(t) = \left(\frac{\pi a}{k_B T}\right) \langle \Delta r^2(t) \rangle.$$
(3.68)

Thus, from the MSD data, numerically evaluated data points (t_i, J_i) can be obtained, or J(t) can be obtained by fitting a model curve to the experimental values of *MSD vs. t*. For recovering $G^*(\omega)$, Evans developed a numerical method by discretization of the partial derivatives; the following formula is used:

$$\frac{i\omega}{G^*(\omega)} = i\omega J_0 + \left(1 - e^{-i\omega t_1}\right) \frac{[J_1 - J_0]}{t_1} + \frac{e^{-i\omega t_N}}{\eta} + \sum_{k=2}^N \left(\frac{J_k - J_{k-1}}{t_k - t_{k-1}}\right) \left(e^{-i\omega t_{k-1}} - e^{-i\omega t_k}\right).$$
(3.69)

The term J_0 corresponds to the compliance at $t \to 0$, and η corresponds to the viscosity at $t \to \infty$. Both quantities can be determined from extrapolation of the DCM curve at short and long times [110].

One different procedure is to employ $s \rightarrow i\omega$ (analytic continuity) such as show in Eq. 3.68, to obtain the real and complex components of $G^*(\omega)$, where $\langle \Delta r^2(t) \rangle$ could be obtained by fitting a model curve to the experimental MSD curve. For the case of particles immersed in WLM solutions, the experimental MSD curves can be best fitted to a model curve proposed by Bellour *et al.* [111]:

$$\langle \Delta r(t)^2 \rangle = 6\delta^2 (1 - e^{\left(-\frac{D_0}{\delta^2 t}\right)^{\gamma'}})^{1/\gamma'} \left(1 + \frac{D_m}{\delta^2} t\right).$$
(3.70)

Here, $\delta\delta^2$ measures the plateau of $\langle \Delta r 2(t) \rangle vs.t$ curve, D_0 , and D_m are the diffusion coefficients for particles in the solvent at infinite dilution and at long times, respectively, and γ' is a parameter that accounts for the broad spectrum of relaxation times at the plateau. It is common for doing the fittings to leave δ ,

 D_m , D_0 and γ' as free parameters. In this function, we observe three different motion regimes shared by all micellar solutions reported in the literature, regardless of concentration or the temperature. At short times, MSD is almost a linear function of time, i.e., a simple diffusion $\langle \Delta r^2(t) \rangle = 6D_0 t$. At intermediate times, the MSD remains constant for a given time interval (a plateau). Here, the model was built to describe the motion of Brownian particles harmonically bound around a stationary mean position, as a consequence $\langle \Delta r(t)^2 \rangle = 6\delta^2 (1 - \exp[-(\frac{D_0}{\delta^2}t)])$, where the particle's amplitude of the motion, the cage size δ , is related to the elastic modulus G_0 $(\delta^2 = k_B T / 6\pi a G_0)$, which does not depend on ω . At these intermediate times, particles are bound to their mean position, on time scales smaller than the micellar system's longest characteristic time. At the inflection point, $6\delta^2$ is the value of the mean square displacement. In a WLMs fluid, the cage where particles are momentarily trapped fluctuates due to the breaking/reptation process. Then, at longer times, MSD is again a linear function of time, $\langle \Delta r^2(t) \rangle = 6D_0 t$ as a consequence, it was proposed $\langle \Delta r(t)^2 \rangle = 6\delta^2 (1 - \exp[-(\frac{D_0}{\delta^2} t)]) (1 + \frac{1}{\delta^2} t)$ $\frac{D_m}{\kappa^2}t$). However, this last expression did not correctly describe the plateau onset time dynamics because the microspheres' dynamics exhibit a vast time relaxation spectrum. This led to the inclusion of the parameter γ' . Recently, Palmero-Cruz *et al.* [112] described the smooth shortintermediate transition of the MSD curve using random walker simulation. They found that the origin of the smooth transition is a wide distribution of confining cages, and the corresponding ensemble averaged 3D of MSD over all confined particles; the wider the cage distribution, the smoother the transition.

As an example of DWS – microrheology technique, the results of DWS – microrheology performance correspond to the system of WLMs of NaNO₃/CTAB with [CTAB] = 0.1 M, [NaNO₃] = 0.3 M measured at 30 °C. In *Chapter 4,* the characteristics of this system, such as the experimental procedure, are presented in detail. Fig. 3.8a. shows the field correlation function, $g_{(1)}(t)$, obtained from the Siegert relation (Eq. 3.33) through the correlation function of the intensity $g_{(2)}(t)$, which was measured experimentally. The constant β corresponds to the average of the first ten values of $g_{(2)}(t)$ minus one. Figure 3.8b. shows the MSD as a function of time, which is obtained by numerical immersion in equation 3.55. The parameters l^* and l_a were

obtained by the IAD method with values of 0.176 mm and 33.98 mm, respectively. It is important to note the ratios $l^*/L = 0.073$ and $l^*/l_a = 0.005$; the l^*/L ratio shows that the light diffusion condition ($L \le 0.1$ [23,91,94]) is met and that $l^*/l_a \ll 1$ indicates that the sample is transparent as observed experimentally. The MSD of the system exhibits a simple diffusive behavior at short times ($\sim 10^{-6} - 10^{-4} s$), a slightly curved plateau at intermediate times ($\sim 10^{-4} - 10^{-1} s$), and a newly diffusive region at long times ($\sim 10^{-1} - 1.4 s$). This behavior of the DCM is typical of colloidal particles embedded in living polymeric systems [20–22,86,113]. The parameters obtained from the best fit are inserting into Fig. 3.8b.

Figure 3.8. a) Correlation function of the field, and b) MSD from numerical inversion of equation 3.55 for NaNO3/CTAB WLMs system with [NaNO3] = 0.3 M, [CTAB] = 0.1 M at 30 °C; the continuous line corresponds to best fit with Bellour equation (Eq. 3.70), and their free parameters are inserted in the figure.

The viscoelastic spectrum of WLMs was obtained from the MSD data. The first technique uses the logarithmic derivative (Eq. 3.67; Fig. 3.9a.), and the second one uses the compliance approach (Eq. 3.69; Fig. 3.9b.). Both ways of obtaining $G'(\omega)$ and $G''(\omega)$ have a good agreement with mechanical rheometry, being a way to corroborate the validity of the results. However, both forms present fluctuations in $G'(\omega)$ and $G''(\omega)$ at high frequencies ($\omega \ge 10^5 \text{ s}^{-1}$), which is associated with experimental MSD noise at short times ($t \le 10^{-5} \text{ s}$). Additionally, the curves $G'(\omega)$ and $G''(\omega)$ via the compliance present noise at intermediate frequencies, around $\omega \sim 10 - 10^1 \text{ s}^{-1}$, capturing the experimental little of the MSD around $t \sim 1 \text{ s}$. With the mention above, it was concluded that for both techniques, the experimental noise of the MSD at short times impacts noise in the viscoelastic spectrum at high frequencies and that the technique via the

complication is more sensitive to experimental noise. The third technique consists of obtaining $G'(\omega)$ and $G''(\omega)$ through equation (3.67) where the unilateral Fourier transform of the best fit according to the Bellour model (Eq. 3.70; Fig. 3.8b.) was evaluated. Fig. 3.9c. shows $G'(\omega)$ and $G''(\omega)$ in good agreement with mechanical rheometry and the techniques mentioned below. In this case, no fluctuations are observed in $G'(\omega)$ and $G''(\omega)$, providing an advantage of obtaining characteristic lengths, as will be discussed in the next chapter.

Figure 3.9. Linear viscoelasticity spectra obtained by DWS-microrheology for three different methods: a) Logarithmic derivative, b) compliance approach, and c) fitting of MSD experimental data using Bellour equation and subsequently unilateral Fourier transform of it. The system is NaNO3/CTAB with [NaNO3] = 0.3 M, [CTAB] = 0.1 M at 30 °C.
4. Results



GRAPHICAL ABSTRACT

4.1 Linear and nonlinear viscoelasticity of living polymers made of worm-like micelles

The entanglement index, κ , is a valuable tool for understanding the origin of the contribution of the elastic forces from a molecular point of view to the rheological behavior of wormlike micelle solutions. Image from [86].

4.1.1 Abstract

The elastic contribution to the fluid dynamics of wormlike micellar solutions makes these fluids unique due to the distinctive self-assembled micellar network formed by tubular giant structures. The mesoscopic scales of the micellar network related to the degree of entanglement can give guidelines for understanding the origin of elastic forces and their effect on rheological response. To establish a relationship between the elastic contribution to rheological response and mesoscopic structure, different rheological measurements were carried out: 1) SAOS protocol to obtain the relaxation time, τ_R , and elastic modulus, G_0 , 2) flow curves, rotating the internal or external cylinder in a Couette geometry for different measurements times, 3) linear shear banding observation, and 4) LAOS protocol for obtain the nonlinear rheological response. All of them to determine how elastic forces modify the rheological behavior in systems made of different ratios of hexadecyltrimethylammonium bromide (CTAB)/sodium salicylate (NaSal) and different ratios of CTAB/NaNO₃. DWS-microrheology was also performed to measure the mean square displacements of microspheres embedded in the micellar fluids to obtain their high-frequency

viscoelastic spectra. With this information, the entanglement index, κ as the ratio of the total contour of the micelles to the entanglement length, was estimated and correlated with the rheological behavior. This work discusses the relationship between the entanglement ratio and the rheological response; a proportionality relationship between both parameters is found; high values of κ are associated with a high entanglement of the network and, with it, interesting phenomena such as multiple shear banding and hysteresis in the flow curves. For low values of κ are not observed.

In the results to be discussed below, the introduction to the system is omitted because it was already done in Chapter 2 (see section 2.2). The linear viscoelasticity spectra, flow curves under different conditions, and linear shear banding observations were made as part of my M.Sc.'s thesis project. The nonlinear rheological study and DWS-microrheology experiments were performed as part of my Ph.D. project. However, the results of the M.Sc. project are included in this Ph.D. thesis because they were part of the discussion of the Ph.D. project that concluded with a full paper publication [86]; the discussion that follows is found in this one.

4.1.2. Experimental section

Materials. Hexadecyltrimethylammonium bromide (CetylTrimethylAmmonium Bromide, CTAB, purity > 99 %, Sigma-Aldrich), sodium salicylate (NaSal, purity 99.5 %, Sigma-Aldrich), sodium nitrate (NaNO₃, purity > 99 %, Sigma-Aldrich). Reagents were used as received. All samples were prepared with ultrapure deionized water (Nanopure, USA). The WLMs solutions where prepared dissolving the powders in deionized water under magnetic agitation overnight at room temperature.

Rheological measurements. Rheological measurements were performed with an MCR-702 TwinDrive rheometer (Anton Paar, Austria), in which two air-bearing supported electrically commuted motors sit opposite each other. Both motors are controlled by the same digital signal processor controller, allowing different testing modes. In the separate-motor-transducer mode, both motors are employed, although synchronized. One motor is kept at a fixed position and managed only as a torque transducer, while the other motor exclusively functions as a drive unit. The linear and nonlinear viscoelasticity spectra were obtained using a Cone-Plate geometry (cone diameter of 49.948 mm, and an angle of 2.007 degrees), while flow curves were obtained using a Taylor-Couette geometry (Inner cylinder diameter of 19.996 mm, and outer cylinder diameter of 22.000 mm).

Linear viscoelasticity spectra (SASO protocol, $G'(\omega)$, $G''(\omega)$ vs. ω) were obtained in a wide frequency sweep, $\omega \approx 1 - 10^2 \text{ s}^{-1}$, at fixed shear strain $\gamma \approx 1\%$ where the viscoelasticity moduli are independent of the strain. Nonlinear viscoelasticity spectra (LAOS protocol, $G'(\gamma)$, $G''(\gamma)$ vs. γ at a fixed frequency) were obtained in a wide shear strain sweep, $\gamma \approx 10^{-1} - 10^3$ %. As mentioned before (see section 3.2), LAOS experiments can explore the full range between steady-state and strongly time-dependent behaviors through two dynamic variables, the Deborah number ($De = \tau_R \omega$) and the amplitude Weissenberg number ($Wi_0 = \tau_R(\omega \gamma_0)$); the variables ω and γ_0 were adjusted. For De ~0.3, viscous behavior is explored, $De \sim 1$ a viscoelasticity behavior, and $De \sim 3$ a solid behavior; in all cases, wide values of Wio are adjusted. The flow curves were developed following a protocol for observing the consequences of rheometric measurements performed faster or lower than τ_{R} . In logarithmic mode, the rheometer spends a measuring time, t^{measuring}, executing a measurement depending on the shear rate according to the equation $t^{measuring} = b \cdot \gamma^m$, where b and m are constants determined by the experimenter. First, select the number of required points to be measured in a whole shear flow experiment, then select the shear rate and time spent for the first and last measurements. The ratio $t^{rel} = t^{measuring}/\tau_R$ determines how long the measurement time is relative to the relaxation time; it is like the inverse of the Deborah number. In addition, the internal or external cylinder of the Couette geometry is rotated while another cylinder maintains static.

Observation of shear banding phenomena. Shear banding observations were performed using a homemade instrument developed in the Complex Fluids group at UNAM: a transparent Taylor-Couette geometry with two concentric quartz cylinders (50 mm in height). The external cylinder (*I. D. 80 mm/O. D. 85 mm*) rotates thanks to an electrically controlled motor, and the inner cylinder (*I. D 70 mm/O. D. 75 mm*) is fixed and filled with water coming from a thermal regulated circulatory bath for thermal control (see Fig. 4.1). The gap between cylinders is 2.5 mm wide. The fluid in the gap is visualized with the aid of a CCD camera (Hamamatsu Vidicon, Japan) and a zoom

lens focused on a sheet of light in the gradient-vorticity plane made with a Ne–He laser beam (Coherent Inc., USA) and a combination of spherical and cylindrical lenses.



Figure 4.1. a) Experimental setup for shear banding observation (image extracted from [12]). b) Frontal image of Taylor Couette geometry design in Complex Fluids Group at Physics Institute, UNAM.

Mesoscopic scales of the micellar network obtained by DWS micro-rheology. As mentioned above, the micellar network mesoscopic scales of interest in WLMSSs solutions that can determine their rheological behavior are the micelles' total contour length, L_c , persistence, l_p , and entanglement, l_e , lengths, as well as the mesh size of the entangled micellar network, ξ . The last chapter presented the experimental methodology (see sections 3.2 and 3.3). All measurements were realized using polystyrene microspheres (*Diam. = 784 nm*, $\phi \sim 0.03$; Bangs Labs, USA), used as tracers, and dispersed in the WLMS solutions. Samples with dispersed particles were deposited in rectangular glass cells (Sterna Cells, USA) with an optical path length of $\sim 2 mm$ and allowed to relax for two weeks before measurement. The particle weight concentration was obtained by thermogravimetry (TGA, TA Instruments, USA); here, the evaporation of the solvent in particle solution at a constant temperature was followed.

4.1.3. Results and Discussion

SAOS and flow curves. As explained below (see section 3.3), G_0 and τ_R parameters were extracted from viscoelastic spectra of micellar solutions at low and intermediate frequencies. For CTAB/NaSal solutions with R = 2 and at R = 4, ([CTAB] = 0.1 M, R = [NaSal]/[CTAB]), measurements were made at different temperatures (20 – 40 °C), and for CTAB/NaNO₃ solutions at different surfactant concentrations at 30 °C ([CTAB] = 0.1, 0.2 and 0.3 M, [NaNO₃] = 0.3 M). Although the elastic modulus does not change significantly for CTAB/NaSal solutions, it is not the case for τ_R , which is quite different in both systems at temperatures below 30 °C; above this temperature, the difference in τ_R is not significant, as observed in Fig. 4.2. The inset of Fig. 4.2 presents τ_R for the different micelle solutions of CTAB/NaNO₃; in this case, G_0 increases with surfactant concentration. Temperature or surfactant concentration controls the rheological response in WLMs, which is useful to obtain a wide range of relaxation time values.



Figure 4.2. τ_R vs. temperature for micellar solutions of CTAB/NaSal with R = 2 and R = 4 ([CTAB] = 0.1 M). Insert, τ_R vs. CTAB concentration micelle solutions of CTAB/NaNO₃, ([NaNO₃] = 0.3 M) at 30 °C. In some cases, error bars are smaller than the symbols in the figures. Image from [86].

As we will see, the relaxation time differences will make the micellar solutions of CTAB/NaSal with R = 2 and R = 4 behave quite differently below 40 °C. In particular, at R = 2, this solution presents gradient shear banding; this is not the case for R = 4 [12]. CTAB/NaNO₃ solutions present linear shear banding at concentrations above 0.1 M in CTAB [19].

Concerning to flow curves, Fig. 4.3 presents scaled flow curves, $\sigma/G_0 vs. \dot{\gamma}\tau_R$, for thixotropic loops where the shear rate is first ramped up (up-shear curve) to some maximum value, then ramped down (down-shear curve) at different temperatures, T= 20, 25, and 30 °C. Measurements were made by rotating the external or internal cylinders while maintaining the other cylinders' static. The time used to measure each point of the flow curve is different in these flow curves. When the symbols are open $t^{rel} < 1$, the measurements fluctuate significantly, and the flow curves present hysteresis. When the symbols are filled $t^{rel} > 1$, both fluctuations and hysteresis are pretty low. Flow curves are essentially the same when $t^{rel} > 1$, no matter what cylinder is moved, but when $t^{rel} < 1$, flow curves do not coincide. As temperature increases, τ_R is pretty small; therefore, it is not feasible to make rheological measurements with the $t^{rel} < 1$ condition, as it occurs at $35 \, ^{\circ}C$ (not shown). Here, all curves behave the same way as in the long-time measurements. As a corollary, thixotropic loops with low fluctuations and small hysteresis are obtained when times larger than τ_R are used during each measured point. The up-shear and down-shear curves coincide no matter what cylinder is rotating.



Figure 4.3. Flow curves for the CTAB/NaSal micellar solutions with R = 2 made in Couette geometry at different temperatures where the inner or external cylinders rotate while the other cylinder remains static. Up-shear curves (Up) are circles, and down-shear (Down) curves are squares: inner rotating cylinder red symbols, external rotating cylinder blue symbols. The time used to measure each point of the flow curve is different: Full symbols correspond to measurements where $t^{rel} > 1$. On the contrary, empty symbols correspond to $t^{rel} < 1$. Insets: These are the same measurements as in the main figure. Up-shear curves (Up) are circles, and down-shear (Down) curves are squares. Inner rotating cylinder black symbols, external rotating cylinder green symbols; for all the cases $t^{rel} \gg$ 10. Image from [86].

Fig. 4.4 presents scaled flow curves for up-shear and down-shear curves for CTAB/NaSal at R= 4, and in the inset for CTAB/NaNO₃; in all the cases, $t^{rel} > 1$. These curves present neither fluctuation nor hysteresis, and the up and down-shear curves are almost identical; they do not depend on the rotating cylinder. The same behavior is observed in CTAB/NaSal at R= 4 as the temperature varies. Equivalent flow curves to those presented before for $t^{rel} < 1$, as mentioned above, are not feasible because the relaxation times are pretty low, 1.4, 0.59, and 0.28 s for 20, 25, and 30 °C, respectively.



Figure 4.4. Flow curves for the micellar solutions performed at $t^{rel} > 1$: CTAB/NaSal ([CTAB] = 0.1), R = 4Inset: CTAB/NaNO3 at 30 °C. All of them are made using Couette cell geometry. Up-shear curves (Up) are circles, and down-shear (Down) curves are squares. In all curves, the internal cylinder is the rotating one. Image from [86].

From a molecular point of view, for $t^{rel} > 1$, the system has enough time to relax, *i.e.*, micelles have enough time to disentangle. Nevertheless, when there is less than one, there is not enough time for the tubular micelles to disentangle due to the speed of the imposed strain. The micellar network is deformed, so the contribution of elastic forces increases, modifying the flow curves. The more entangled the micellar network, the more significant the micellar disentanglement time. Also, it is possible to observe another feature in this case: when the ratio of elastic to viscous forces is small, *i.e.*, Wi < 1 ($Wi = \tau_R \dot{\gamma}$), do not observe differences in the flow curves for all the cases of the implemented protocol. For Wi > 1, the elastic contribution to the dynamic forces during the flow produces different flow curves, as observed in Fig. 4.3.

Shear banding observation. The external cylinder is rotating in the experimental setup, and the internal one is fixed. It is well known that inertial Taylor instability does not occur when the outer cylinder is rotating for Newtonian fluids [18]. In contrast, instabilities do not depend on which cylinder is rotating in the purely elastic analog [114]; however, below for a critical value of dimensionless curvature of the streamlines ($\Lambda = d/R$), shear banding does not show secondary flow instabilities [115]. In this case, $\Lambda = 0.066$, which is smaller than the critical value of Λ . Therefore, we expected only to observe an instability because the WLMS solutions become inhomogeneous

close to $Wi \sim 1$ and $\sigma/G_0 \sim 1$. Along the flow curve plateau, where the fluid becomes inhomogeneous, scattered light coming off a page of light in the gap of a Couette cell geometry (gradient-vorticity plane) made of two transparent concentric quartz cylinders allows the observation of gradient shear bands because of nematic phases scatter light much more than an isotropic phase.

Thixotropic loops were developed, giving long or short times for deformation to the fluid with respect to τ_R ($t^{rel} \gtrsim 1$ or $t^{rel} \lesssim 1$). Typical images of what is observed in the gap in the gradient-vorticity plane can be seen in Fig.5a-5b for WLMS solutions of CTAB/NaSal for R = 2 and 25 °C. In all cases, a paranematic phase appears close to the moving cylinder (external one) in the up-shear curve at $Wi \sim 1$; as the shear rate increases, more bands are formed. Although curves are relatively more stable for $t^{rel} \gtrsim 1$, the paranematic phase is pretty dynamic, forming and reforming themselves in different positions along the gap, wriggling, and changing their shapes. After several experiments, there is no significant difference if t^{rel} is greater or lesser than one. The scattered intensity is constant on the average along the gap at a fixed shear rate; the average increases as the shear rate increases. As the Wi number increases, bands are thicker; most of the time, there is not just one band. The contrast between isotropic fluid and the paranematic fluid (dark and brighter regions) seems higher after a long time. However, it is certainly challenging to find clear trends or differences. The conclusion is that the position where bands are formed and their size are random.

Image processing provides spatiotemporal characteristics of the observed bands during the shear banding. In the central part of the gradient-vorticity plane, from video recordings of the bands, a light intensity distribution I(x) from a horizontal line along the gradient direction was sampled at regular time intervals to produce space-temporal diagrams I(x,t) of the flow pattern. These space-time plots are helpful for determining the onset and evolution of flow transitions and instabilities. Fig. 5c presents an example of a space-time plot for CTAB/NaSal WLMS solutions for R=2 and 25 °C, where some features are relatively straightforward. At $t^{rel} \gtrsim 1$, the contrast between paranematic bands and isotropic fluid is lower, revealing fewer fluctuations and more stable bands, although it is not common to observe just one band. At $t^{rel} \leq 1$, there are also many bands, and light scattering is more intense in the paranematic and isotropic fluids. The flow is suspected to be less stable due to the increase in fluctuation. Experiments with CTAB/NaSal for R = 4, do not show shear bands. For CTAB/NaNO₃ with [CTAB] = 0.2 and 0.3 M, presents just one band close to the moving cylinder (not show) in good agreement with the literature [24]; this phenomenology is called simple shear banding scenario.



Figure 4.5. Gradient shear banding in CTAB/NaSal WLMS solutions for R=2 and 25 °C. Flow curves σ *vs.* $\dot{\gamma}$ for thixotropic loops: a) for $t^{rel} < 1$, and b) for $t^{rel} > 1$. The fluid in the gap is observed with a page of light in the gradient-vorticity plane (the color is artificial). The images are observations approximately where the arrows are pointing out. c) Space-time plot grayscale images: Horizontal light intensity on a line of pixels along the gradient direction (vertical) vs. time (horizontal) for CTAB/NaSal WLMS solutions, for R = 2 during thixotropic loops. Bright regions correspond to the paranematic fluid. They correspond to the images in (a) and (b) at some particular time. The external cylinder is rotating (bottom of the spacetime graphs), and the inner cylinder is fixed (top of the space-time graphs). Image from [86].

Large Amplitude Oscillatory Strain. LAOS strain-imposed protocol was performed on the WLMS solutions under study, which follow the Maxwell model at low and intermediate frequencies when the applied strain is small $\gamma_0 \ll 1$ (SAOS protocol). As mentioned above (see sections 2.3 and 3.3),

the shear stress in the Maxwell model for an oscillatory shear strain of amplitude $\gamma_0 = Wi_0/De$ and angular frequency $\omega = De/\tau_{R_i}$ can be written as $\sigma(t) = (Wi_0/De)[G'\sin(De/\tau_R)t +$ $G'' \cos(De/\tau_R) t$], where $G' = G_0 [De^2/(1 + De^2)]$ and $G'' = G_0 [De/(1 + De^2)]$. The loci of $\sigma(t)$ vs. $\gamma(t)$ for this model is an ellipsis given by $\sigma^2 - 2G'\sigma\gamma + (G'^2 + G''^2)\gamma^2 = [G''Wi_0/De]^2$, where the stress maximum in a general linear viscoelastic material always occurs in the first quadrant (all variables positive) of a σ vs. γ graph ($\sigma^{max} = [(G'^2 + G''^2)/G']\gamma$). The response of WLMs solutions to oscillatory shearing strain is probed by selecting γ_0 , which in LAOS is not limited to small values, producing deviations from the linear behavior. For a fixed Wi_0 value, when $De \gg$ 1, WLMSs solutions can display a nonlinear elastic response at frequencies whose periods are shorter than the time scale needed to form shear bands, particularly when $Wi_0/De > 1$. Generally, when the ratio of relaxation time to the time scale of the deformation observation ($De \ll 1$) is quite small, a reconfiguration dynamic that keeps pace with the applied deformation is expected. This flow leads to a quasi-steady state response, in which the stress slowly sweeps up and down as the shear rate varies through a cycle. Time-resolved small-angle neutron scattering t-SANS in a WLMS solution [116], similar to those studied here, have identified different frequency regimes matching nearly perfectly the expectations of the linear viscoelastic Maxwell model.

In Figs. 4.6 – 4.8 shows Pipkin diagrams: 3D Lissajous-Bowditch curves $(\sigma/\sigma_0 \ vs. \gamma/\gamma_0 \ vs. \dot{\gamma}/\dot{\gamma}_0)$ and their projections (elastic representation: $\sigma/\sigma_0 \ vs. \gamma/\gamma_0$, and viscous representation: $\sigma/\sigma_0 \ vs. \dot{\gamma}/\dot{\gamma}_0 \ (= \dot{\gamma}/\omega\gamma_0))$ at different values of *De* and *Wi*₀. Here, γ_0 and σ_0 are the maximum shear rate and maximum shear stress in an oscillation cycle used to scale the shear stress and shear rate, respectively. In the mentioned figures, rows are arranged for different $De(=\tau_R\omega)$ values, *i.e.* varying the frequency of shear oscillation; then, in a row, we present on the left results for $De \sim 0.3$, in the center for $De \sim 1$, and on the right for $De \sim 3$. Relaxation time varies due to shifting the surfactant concentration (Fig. 4.6) or temperature (Figs. 4-7 and 4.8).

The top rows correspond to large τ_R values, which decrease downward. The 3D Lissajous-Bowditch curves and their projections are more or less similar for each system going down along a specific column because graphs are scaled variables. In contrast, more significant behavior changes are observed in all the systems going from left to right along a row. Here, moving rightward along a row in these figures, the system's relaxation dynamics cannot keep pace with

the applied deformation. In these graphs, the amplitude of shearing strain, $\gamma_0 = Wi_0/De$, varies, depending on the case, from a little less than one to $\gamma_0 \sim 10$ (curves with a different color). Fig. 4.6 presents the CTAB/NaNO₃ micellar solution, the 3D Lissajous-Bowditch curves, and their projections. As mentioned, this system presents shear banding in steady shear for the concentration above 0.1 M in CTAB (systems in the two upper rows of Fig. 4.6). For $De \sim 0.3$, for $Wi_0/De \le 1$, linear viscoelasticity is observed since the shape of the $\sigma/\sigma_0 vs. \gamma/\gamma_0$ curves are ellipsoidal in the elastic representation; their maximum stress is also in the first quadrant of this representation. On the contrary, as $Wi_0/De > 1$, the ellipsoidal shapes are lost, and the stress maxima move to the second quadrant in the elastic representation, *i.e.*, their behavior is nonlinear. For $De \sim 1$, the behavior is the same as for $Wi_0/De \leq 1$, the σ vs. γ curves describe ellipses; but when $Wi_0/De > 1$, the curves show a behavior more nonlinear than in the previous case, except for the case of the lower CTAB concentration (lower row, [CTAB] = 0.1). Here, the system's behavior seems viscoelastic since the σ vs. γ curves describe elliptical shapes in the elastic representation. For De \sim 3, the behavior is viscoelastic for most solutions. However, for the case of the solution with the largest CTAB concentration, as the Wio/De increases over one, the curves in the elastic representation become less ellipsoidal, deviating from the Maxwell model to showing a clearer nonlinear behavior. For the lower CTAB concentration case ([CTAB] = 0.1), the behavior of the σ vs. γ curves correspond to a linear elastic response, as observed in the elastic (forming almost a diagonal line) and viscous representations (forming almost a circle). The elastic contribution makes the fluid behavior more nonlinear as more CTAB is added to the solution, which is also the direction of increase in τ_R .

From the mesoscopic point of view, as CTAB concentration increases, the total WLMS contour length increases, as well as the degree of entanglement of the micellar network, as we will show below. This is one of the underlying reasons for the increase of an elastic contribution on the forces involved in the flow and the nonlinear response. The behavior described for the CTAB/NaNO₃ system is similar to that described by the Lissajous–Bowditch curves in LAOStrain given by the nRP model with a nonmonotonic constitutive behavior [16].



Figure 4.6. Normalized 3D Lissajous-Bowditch curves and their projections (elastic representation: $\sigma/\sigma_0 \ vs. \gamma/\gamma_0$, and viscous representation: $\sigma/\sigma_0 \ vs. \dot{\gamma}/\omega\gamma_0$) for CTAB/NaNO₃ micellar solutions at different $Wi_0 (= \tau_R \omega \gamma_0)$ and $De(= \tau_R \omega)$. Surfactant concentration from the upper row to the lower one: [CTAB] = 0.3, 0.2, and 0.1 M, respectively; [NaNO₃] = 0.3 M, and T = 30 °C. Image from [86].

Fig. 4.7 shows the 3D Lissajous-Bowditch curves and their projections for CTAB/NaSal for R = 4. In this WLMS solution, shear banding in steady shear is never observed when the temperature is varied [12]. Although the Lissajous–Bowditch curves and their projections are not equal to the case of CTAB/NaNO₃, they are relatively similar. In all the cases with $Wi_0/De \le 1$, the system follows

a viscoelastic linear flow because the $\sigma/\sigma_0 vs. \gamma/\gamma_0$ curves describe an ellipse, and the stress maxima are in the first quadrant in the elastic representation. However, when $Wi_0/De \ge 1$, WLMs solutions do not follow the ellipsoidal shape, revealing a nonlinear behavior. In $De \sim 3$, although the behavior is nonlinear, the elastic contribution seems to be more important than in $De \sim 0.3$, because, in the elastic representation, the curves present relatively more elongated diagonal shapes and relatively more circular shapes in the viscous representation. This is particularly clear in the direction where τ_R decreases (higher temperatures), and entanglement decreases, as we will see below. In agreement with the LAOStrain results given by the nRP model with a monotonic constitutive curve [12,117], expect that shear banding could appear during the experimental development of the Lissajous-Bowditch curves at some specific shear deformations.

In contrast with the cases presented in Fig. 4.6, and 4.7, Fig. 4.8 shows very different results for the CTAB/NaSal micellar solution for R = 2. This system presents gradient shear banding under steady flow, but as reported before [12], and discussed below, the bands do not show a simple scenario; several bands are observed. In general, in all the cases of this micellar system with $Wi_0/De \le 1$, a viscoelastic linear flow is observed because the $\sigma/\sigma_0 vs. \gamma/\gamma_0$ curves describe elliptical shapes, and the stress maxima are in the first quadrant in the elastic representation. For $Wi_0/De > 1$, different behavior is followed going to the right in a row. At $De \sim 0.3$, the response of WLMs solutions to oscillatory shearing strain is nonlinear and going downward in the figure (higher temperatures and lower τ_R), there is a significant increase in the shear stress, which are stress overshoots. As the *De* number increases, these stress overshoots are not so prominent at particular τ_R ; they almost disappear at $De \sim 3$.



Figure 4.7. Normalized 3D Lissajous-Bowditch curves and their projections (elastic representation: $\sigma/\sigma_0 \ vs. \gamma/\gamma_0$, and viscous representation: $\sigma/\sigma_0 \ vs. \dot{\gamma}/\omega\gamma_0$) for CTAB/NaSal micellar solution for R = 4, at different $Wi_0 (= \tau_R \omega \gamma_0)$ and $De(= \tau_R \omega)$. Temperature from the upper to the lower row T = 20, 25, and 30 °C, respectively; [CTAB]= 0.1 M. Image from [86].

At $De \sim 0.3$, the ratio of relaxation time to the time scale of deformation observation is relatively small, and we observe a quasi-steady response that must be in some way similar to what is observed in a steady flow, as described above. In the elastic or viscous representations, we observe significant oscillations. We suspect that the oscillations could be due to the formation of multiple bands when the system reaches that region of the cycle, as it occurs during steady shear flow, as shown in the next section. As far as $De \sim 1$ with $Wi_0/De > 1$, the nonlinear behavior is substantial, but there are no oscillations as in $De \sim 0.3$. However, at $De \sim 3$ with $Wi_0/De > 1$, the system behaves nonlinearly in the upper rows (lower temperatures and larger τ_R), but the system becomes relatively more viscoelastic in the lower ones. Here, τ_R is smaller, as well as entanglement.

It is important to note the following fact after depicting the 3D Lissajous-Bowditch curves and their projections for three different WLMS solutions, which, due to the temperature or surfactant concentration, present different τ_R values. Despite being scaled Pipkin diagrams, systems for the same *De* and *Wi*₀ numbers in a column do not reflect a scaled invariance; some curves corresponding to the same color are similar but quite different in other cases. It is suspected that is due to the degree of entanglement of the micellar network, which is responsible for elastic forces and different for each WLMs solution but not considered in the description yet. The micellar network needs to be disengaged during the LAOS protocol. In particular, at high frequencies, as in the case of *De* ~ 3 with *Wi*₀/*De* > 1, systems with large τ_R cannot be disentangled efficiently; then, their degree of nonlinear behavior is relatively large.



Figure 4.8. Normalized 3D Lissajous-Bowditch curves and their projections (elastic representation: $\sigma/\sigma_0 vs.\gamma/\gamma_0$, and viscous representation: $\sigma/\sigma_0 vs.\dot{\gamma}/\omega\gamma_0$) for CTAB/NaSal micellar solution for R = 2, at different $Wi_0(=\tau_R\omega\gamma_0)$ and $De(=\tau_R\omega)$. Temperature from the upper to the lower row T = 20, 25, 30, and 35 °C, respectively; [CTAB]= 0.1 M. Image from [86].

Relationship between mesoscopic parameters and dimensionless elasticity number. The main mesoscopic scales that characterize the micellar network were characterized according to section 3.3 (experimental procedure) and section 2.3 (theoretical framework), where the example shows there correspond to WLMs made with NaNO₃/CTAB con [CTAB] = 0.1 M, [NaNO₃] = 0.3 M at 30 °C. The results for different surfactant concentrations and temperatures of the solutions discussed here are in Table 1. These quantities change when the amount of surfactant, the ratio of salicylate to surfactant, temperature, or ionic strength of the media vary.

Characteriz									
Parameter	τ _R (s)	G ₀ (Pa)	G'' _{min} (Pa)	$\omega_0 (s^{-1})$	ξ (nm)	l _p (nm)	l _e (nm)	L _c (nm)	К
									$=L_{Q}/I_{e}$
[CTAB]	NaNO ₃ /CTAB, [NaNO ₃] = 0.3 M a 30 °C								
0.1 M	0.19	24.2	4.9	14378	119.1	35.7	265.8	1974.4	7.4
0.2 M	0.36	56.5	9.8	30313	89.7	27.9	195.6	1745.2	8.9
0.3 M	0.26	66.4	10.1	55663	85.0	22.7	204.7	2159.3	10.5
T (°C)	NaSal/CTAB, [CTAB] = 0.1 M y R = 4								
20	1.91	45.3	4.2	32932	95.5	24.8	234.4	4611.2	19.7
25	0.64	39.7	5.9	58825	100.4	21.4	281.3	3018.1	10.7
30	0.57	30.7	5.1	146378	109.9	15.3	409.3	3845.4	9.4
T (°C)	NaSal/CTAB, [CTAB] = 0.1 M y R = 2								
20	4.85	34.3	2.00	103636	104.7	17.0	352.5	12416.3	35.2
25	2.30	37.2	2.6	63322	102.5	20.1	303.8	8271.7	27.2
30	0.58	36.3	4.5	45665	104.0	22.5	288.1	3921.6	13.6
35	0.16	38.8	6.3	15620	102.3	36.2	204.6	1967.2	9.6

Table 4.1. Microrheological parameters (τ_R (s), G_0 (Pa), and G''_{min} (Pa)), and the mesoscopic scales that characterize the micellar solutions' micellar network.

In WLMs solutions, the *Wi* number takes the role of a control parameter in steady shear flow, which compares elastic to viscous forces in the fluid, as shown in the scaled curves (σ/G_o vs. $\dot{\gamma}\tau_R$) shown above (see Fig. 4.3 – 4.4). When *Wi* < 1, the fluid's elastic to viscous forces ratio is small. Apparently, the micellar network is under a slowly disentangling viscous flow since the deformation rate is slow. When *Wi* ~ 1, there is a regime change from linear to nonlinear. Below a scaled critical temperature in a master dynamic phase diagram, in which an isotropic and a

paranematic phase coexist [62], the fluid under shearing onsets the separation into two sectors, the fluid shear thins, and the $\sigma/G_0 vs. \dot{\gamma}\tau_R$ curve presents a stress plateau. As Wi grows up above one, elastic forces in the fluid are more significant than the viscous forces since viscosity decreases as $\dot{\gamma}$ increases along the coexistence region. Fig. 4.9a presents *El vs. Wi* for the micellar solutions studied here. As mentioned above (see section 3.3), the elasticity is defined as the ratio of viscoelasticity forces and inertial forces, i.e, El = Wi/Re, where Re is the Reynolds number which was calculated for a Taylor-Couette geometry ($Re = \frac{\rho d^2 \dot{\gamma}}{n}$; ρ is the density of the solution, d is the geometry gap, and n the viscosity of the solution). At Wi < 1, inertial forces are negligible compared to those related to elastic deformations suffered by the micellar network. El is relatively constant here and can reach several orders of magnitude depending on the system. When $Wi \sim 1$, El starts to decay linearly following the instantaneous viscosity observed when Wi > 1, and El can decrease more than one order of magnitude in some systems (CTAB/NaSal), although their El values can still be pretty significant. The trend of *El* is similar to that followed by τ_R given for all systems in Fig. 4.2. The isotropic-paranematic transition is observed in CTAB/NaSal solutions with R = 2 at very high El values. The CTAB/NaSal solutions with R = 4 also have high *El* values; however, they are not as high as R = 2 but do not present that phase transition. For the case of CTAB/NaNO₃, where EI values are orders of magnitudes below those of the CTAB/NaSal systems, the shear banding transition is present just for the larger elasticity values. Nevertheless, it does not appear for the lowest El value. More cases need to be studied to determine if there is a tendency to find shear banding when El grows.

The *Re* and *Wi* numbers are ratios between different forces involved in the flow of a fluid, which also depends on the flow conditions. However, they are not measuring directly any intrinsic property related to the elasticity of the micelle network that is perturbed when the fluid slowly flows. This is particularly obvious when Wi < 1, where inertial forces are negligible due to the low speed of the fluid flow, concerning those related to elastic deformations suffered by the micellar network, which is slowly disentangling during that deformation. Here, the mesoscopic scales L_c and I_e could be of help. The ratio between L_c and I_e can be evaluated as the number of entanglements that an average micelle has close to equilibrium by measuring the mesoscopic scales of a micellar network as presented above. It would be pretty reasonable that as there are

more entanglements per micelle, the micelle network could react more elastically to any slow deformation while trying to disentangle. Therefore, it is possible to define an entanglement index, $\kappa = L_C/l_e$, for WLM solutions; this ratio explains in some micellar solutions the viscosity dependence on temperature because entanglement decreases with increasing temperature, which also explains why the elasticity number measured in Couette flow decreases when the temperature increases [22,113]. Recently, other authors have started to use this parameter to $G_0/G_{min}^{\prime\prime}$ to measure the entanglement [118], which is close to κ definition. Fig. 4.9b shows that when κ increases, EI also increases nonlinearly; a big shoulder appears at $\kappa \sim 10$, apparently separating two regimes. One possible explanation as to why for $\kappa > 10$ the elasticity decreases is a shear thinning effect; as κ increases, the elasticity of the micellar network increases, and its disentanglement becomes more difficult, so chains must partially align to facilitate their flow under an imposed deformation, reducing their viscosity and thus their elasticity. However, the last surmise is a hypothesis, and from a molecular point of view, it is not clear whether these regimes could be related to a change of contribution between inertial and elastic forces to El; further theoretical development is needed to understand this feature. The large values in κ are the underlying reason for observing in some WLM solutions so large values of El at small values of Wi; this is the case of CTAB/NaSal solutions with a significant κ value, in contrast with those of CTAB/NaNO₃ where κ is much lower.



Figure 4.9. a) *El vs*. $Wi(=\dot{\gamma}\tau_R)$ for the micellar solutions of CTAB/NaSal ([CTAB] = 0.1 M), for R=2 and R = 4, at different temperatures, and of *CTAB/NaNO*₃ ([*CTAB*] from 0.1 to 0.3 M, and [*NaNO*₃] = 0.3 M), at 30 °C. b) *El vs*. κ for the WLM solutions, where *El* is calculated along the vertical red line showed in (a), for a Couette cell with d = 2.5 mm, $R_i = 37.5 \text{ mm}$, $\Lambda = 0.066$. Image from [86].

Flow curves are essentially the same in thixotropic loops when $t^{rel} > 1$, as discussed above, no matter what protocol or cylinder of the Couette geometry is rotating. Although the flow strains the micellar network, there is enough time for a slow disentangling. Nevertheless, when $t^{rel} < 1$, flow curves do not coincide and present large fluctuations because there is no time for the micellar network to be disentangled, so the response to the strain of the micelle network in the fluid is quite elastic and nonlinear. Thixotropic loops that present multiple bands correspond to micellar solutions with a significant degree of entanglement, as is the case for CTAB/NaSal with R = 2. However, CTAB/NaSal with R = 4 does not show shear bands, where κ is lower than for R =2. In contrast, for CTAB/NaNO₃ solutions, κ is relatively low, and when they present banding, it is just one band close to the moving cylinder (simple scenario). It is unclear why CTAB/NaSal with R = 4 does not present shear banding with such a high entanglement. Probably, we are extrapolating to the concept of κ , which was measured in equilibrium, and there is another component related to the dynamics of the shear banding formation playing an important role.

As mentioned above, in the Lissajous-Bowditch curves, a specific column, τ_R , is different going downwards. Pipkin diagrams in scaled variables for the same *De* and *Wi*₀ numbers do not reflect scaled invariance. Just curves corresponding to the same color where *Wi*₀ < 1 are almost superimposable. However, when $\gamma_0 = Wi_0/De$ increases, the micellar network is highly deformed, which is harsher in LAOS at high frequencies because micelles do not have enough time to disentangle at the speed of the imposed strain. Then, the larger κ , the more nonlinear behavior will be expected. However, in LAOS experiments, we cannot evaluate κ along the deformation steps in the experiment in the present state of our knowledge because these experiments are developed far from equilibrium as the frequency increases. The index κ is obtained in equilibrium conditions, and it cannot be used as *Wi* or *Wi*₀ notoriously increase because the micellar network is happening in the system by extrapolating what is occurring at low values of *Wi*. As mentioned above, we suspected the degree of entanglement of the micellar network, which is responsible for elastic forces, must be substantial. This seems to be correct from the data in Table 4.1. For the

CTAB/NaNO₃ solutions, as the CTAB concentration increases, the micellar length does not vary so much, although it increases at a higher concentration. However, l_e slightly decreases as the CTAB concentration increases; therefore, the degree of entanglement, κ , increases. In the CTAB/NaSal solutions with R = 4, l_e decreases as temperature drops; L_C first decreases with temperature but increases at 25 °C. Hence, the degree of entanglement given by κ increases as temperature decreases. Finally, in the case of CTAB/NaSal with R = 2 solutions, L_C notoriously increases when the temperature decreases, and l_e also increases, but at a slow pace. Therefore, the entanglement notoriously increases. The micellar network can slowly be disengaged during the LAOS protocol at large γ_0 and low frequencies but not at high frequencies (De ~ 3 with $\gamma 0 = Wi_0 / De > 1$). Micellar solutions with large κ cannot be disentangled efficiently; their degree of nonlinear elastic behavior is relatively large. It is essential to mention that the physicochemical parameters of micellar solutions determine the mesoscopic scales; apparently, the other mesoscopic scales also change, but they do not clearly impact the rheological behavior as $\kappa = L_C / I_e$.

Finally, it is essential to mention that the disentanglement mechanism is unknown. Several mechanisms are probably occurring at the same time. Micelles could slide or reptate to leave the entanglement through viscous flow, break and reform, or through the transient character of crosslinks, where when a micellar tread collides or contacts an entanglement, it forms a transient crosslink that recombines on the other side of the encountered topological constraint (ghostlike crossing).

4.1.4. Conclusions

In this study, different experiments (flow curves, small and large oscillatory shear tests, linear shear banding observations) were done to determine how elastic forces modify the rheological behavior of Maxwellian fluids due to the contribution of elastic forces to the flow. Also, we made diffusive wave spectroscopy micro-rheology to obtain the mesoscopic length scales of the entangled micellar network in WLM solutions, which are determined by the solutions' physicochemical parameters (concentration of surfactants, hydrotropic salts, temperature, the ionic force of the media, etc.). Understanding the origin of how the elastic forces affect the rheological behavior of micellar fluids is not done by proposing a model and solving it for different

experimental protocols, as mainly made for LAOS experiments in Refs. [16,17]. The approach was to find a property that somehow measures entangling in the mesoscopic micellar network, giving rise to elastic forces opposing fluid deformation during flow. This property is the entanglement index, κ , which allows us to understand the consequences of the entanglement in the experiments we performed, explaining why different micellar systems are ordered in the way shown in the diagram *El vs. Wi* of Fig. 4.9a in terms of the characteristic length scales of the supramolecular structures, which self-assembles in the fluid. Mesoscopic simulation methods for predicting the rheology of WLMs [16,119] need to be done in the future to confirm the correlation between *El* and κ , as observed in Fig. 4.9b, and to determine if the apparent two regimes observed in that figure have some physical insight.

4.2 Linear and nonlinear viscoelasticity of flexible biopolymer gels



GRAPHICAL ABSTRACT

Depending on the gelation mechanism, biopolymer gelatin gels are commonly classified as physical, chemical, or hybrid; in hybrid gels, the physical and chemical crosslinking mechanisms occur simultaneously. The chemical and physical contribution in the network can weigh the fractal dimension. Image from [120].

4.2.1 Abstract

Depending on how they form their linkings, biopolymer gelatin gels are commonly classified as physical, chemical, or physicochemical (in this work, sometimes called hybrid); in gelatin hybrid gels, the physical and chemical crosslinking mechanisms run simultaneously. This work explores how different gelation mechanisms affect the network structure through a rheological and microrheological approach using gelatin molecules as a model of flexible polymer. Chambon & Winter criteria was used, using SAOS protocol to determine the rheological behavior close to the gel point. The strain-hardening behavior of matured gels using the LAOS protocol was investigated to determine the fractal dimension, and the BST-scaling model was employed to interpret the results. Additionally, we obtained the Lissajous-Bowditch curves like a fingerprint that characterized the different types of gels. Hybrid gels' fractal dimension is between 1.46 and 1.60, where the value depends on the dominant crosslinking process.

The gelation kinetics process is followed by measuring the mean squared displacement of microspheres embedded in gelatin solutions using DWS, which in turn allows evaluating $G'(\omega)$ and $G''(\omega)$, the persistence length, and the mesh size as a function of time along the gelation process.

The MSD, as a function of the elapsed time from the starting gelation process, follows a behavior that depends on the gelation processes. As time elapses after gelation starts, the persistence length of the unstructured, non-bonded flexible polymer sections decreases due to the formation of bonds. In the hybrid case, it is not a mixture of both processes; they are not independent when running simultaneously. The time evolution of the gel network's mesh size roughly follows an exponential decay. The results will be discussed below; the introduction to the system is omitted because it was already done in Chapter 2 (see section 3.3). The discussion that follows was published as a full paper [120].

4.2.2. Experimental section

The rheological and microrheological measurements were performed for three different gels: physical, chemical, and hybrid. The network formation is achieved by resrtucturing from single-strand to triple-helix gelatin chains in physical gelatin gel. To form physical crosslinking, the temperature decreases below gelation temperature, T_g ; the coils randomly transform into partially renatured intermingled ordered triple helices mainly held together by hydrogen-bond junctions. If the temperature increases again, the triple helix conformations return to the coiled state, and the gel reversibly melts into a solution [30,36]. For chemical gel, chemical reactions form the network where permanent covalent bonds are created. Consequently, gelation is irreversible; in this work, glutaraldehyde was used as a chemical crosslinker. Furthermore, it is feasible to form hybrid gelatin gels by mixing crosslinking processes, *i.e.*, physical and chemical, producing the so-called physicochemical gel formed by adding a chemical cross-linker agent and decreasing the temperature below T_g . Fig. 4.10 shows a carton of these gels according to crosslinking processes.



Figure 4.10. Cartoon with three gelatin types: physical, chemical, and physicochemical gels with glutaraldehyde as the crosslinking agent. T_g is the gelation temperature. Image from SI of [120].

Materials. Gelatin from porcine skin (type A, gel strength 300 Bloom, Mol. Wt. 87,500 Da, Sigma-Aldrich USA), glutaraldehyde solution ($C_5H_8O_2$, Sigma-Aldrich Germany), sodium azide (NaN₃, purity > 99 %, Sigma-Aldrich USA), and hydrochloric acid (HCl, Sigma-Aldrich USA). Solutions were prepared with ultrapure deionized water (Nanopure, USA). Chemical products were used as received. At low pH, glutaraldehyde molecules react through a hemiacetal bond with two hydroxyproline residues [121], which are linked to the gelatin chain through a covalent peptide bond. For describing the chemical and physicochemical gels, use the parameter R = [Glutaraldehyde]/2[Hydroyproline] to indicate the molar quantity of glutaraldehyde, which will be interlinked with an equivalent molar number of hydroxyproline residues in the gelatin strand; hydroxyproline concentration for gelatin type A is ~13.5 Wt % [121].

To prepare gelatin solutions, gelatin powder was dissolved in ultrapure deionized water under magnetic agitation at $60 \, ^{o}C$ overnight. Before starting the gelation process, the pH of the gelatin solution was adjusted to pH = 4 with [HCl] = 0.1 N.

Rheological measurements. All measurements for SAOS and LAOS were performed with a MCR-702 Twin Drive rheometer (Anton Paar, Austria) using a cone-plate geometry (2° cone angle, Diam. = 40 mm) and temperature control ($\pm 0.1 \text{ }^{\circ}\text{C}$). A solvent trap was used to avoid water evaporation.

Estimation of the gelation temperature. Viscoelastic spectra with SAOS protocol were obtained in a range of $\omega \approx 0.1 - 350 \text{ s}^{-1}$, with a constant strain of $\gamma \sim 0.8 \%$, for different gelatin solution concentrations, C (= 5 - 16 Wt %). Measurements started at high temperatures ($T \sim 50 \text{ °C}$) where the rheological behavior of the solutions is liquid-like ($G''(\omega) > G'(\omega)$), with previous thermal equilibration ($\geq 1 h$). Then, the temperature was systematically lowered for subsequent SAOS measurements until $G'(\omega) \sim G''(\omega)$ over a wide frequency range to reach the gelation temperature (T_g). The rheological behavior for $T < T_g$ is solid-like ($G'(\omega) > G''(\omega)$).

Estimation to reach the gel-point time and viscoelastic spectra at the gel point. An estimation of the gelation time is given by the time needed to reach the gel point, *i.e.*, the elapsed time to reach $G'(t) \sim G''(t)$ along an isothermal time sweep experiment with a constant frequency $(\omega = 0.5 \text{ s}^{-1})$ and constant strain ($\gamma = 0.8 \%$). The viscoelastic spectra are performed after a thermal quench, where the sweep is performed below the initial starting solution temperature (T = 35 °C). The measurement of $G'(\omega)$ and $G''(\omega)$ started after a specific elapsed time, close to the gelation time. For physical and physicochemical gels, using different quenches at $T \leq T_g$, the viscoelastic spectra can be determined very close to the gel point, where $G'(\omega)$ and $G''(\omega)$ are parallel (at constant strain, $\gamma = 0.8 \%$) in a wide range of frequencies. For chemical gels made by glutaraldehyde addition, there is no quenching. All measurements are made at T = 35 °C. The rheological measurements were performed at least two times.

Linear and nonlinear viscoelastic spectra of mature gels. The viscoelastic spectra (SAOS) were obtained on matured gels after 4 h of starting the gelation at some *T*. Frequency sweep measurements were carried out in the range of $\omega = 0.1 - 100 \, s^{-1}$ at $\gamma = 0.8$ %. Afterward, for LAOS measurements, strain-sweep measurements were developed ($\gamma = 0.1 - 5000$ % *at* $\omega = 1 \, s^{-1}$). Lissajous-Bowditch curves were obtained for one imposed intra-cycle shear strain. The rheological measurements were performed at least two times.

Microrheology and diffusive wave spectroscopy. The Mean Square Displacement is measured with DWS according to described in section 3.3. The experimental MSD curves were fitted using the Bellour model [111]. Polystyrene microspheres (dia. 500 nm and 784 nm; Bangs Labs, USA) were added to the solutions (vol. fraction ~ 0.025) at 35 °C, mixed, and placed in a rectangular optical glass cuvette (2 mm optical-path length, Sterna Cell, Inc.). Physical gels are

quenched in a thermal bath to $T \le T_g$. This quenching mark the starting point for the gelation process and the MSD measurements. The MSDs of probe microspheres are determined by collecting their scattered light from a speckle over 180 - 300 s, and the intensity auto-correlation functions are evaluated; measurements were taken along ~ 8 h for different waiting times after gelation started. Scattered light collected for 180 s is enough to consider local thermodynamic equilibrium with good statistics. Protocols for measuring the MSD for chemical and physicochemical gels are similar. An aliquot of glutaraldehyde is added to the gelatin mixture after mixing the microspheres into the gelatin solutions; we considered this moment the starting point of the gelation process for measuring the MSDs. Cuvettes were introduced into a thermal bath for chemical gels at $T(>T_g) = 35$ °C. For physicochemical gel, the thermal bath is at $T \le T_g$ to start the physical bonding simultaneously.

From the MSD, the viscoelastic spectra ($\omega \approx 10^2 \cdot 10^6 \text{ s}^{-1}$) can be evaluated up to high frequencies, and the observation of stress relaxation through Rouse-Zimm and bending modes of the polymer chains is affordable. At those frequencies, $G^*(\omega)$ exhibits a power-law behavior, $|G^*(\omega)| \sim \omega^{\mu}$, with $\mu \sim 5/9$ for Rouse-Zimm modes, which shifts to $\mu \sim 3/4$ where the internal bending modes of Kuhn segments dominate. The change occurs at the frequency, ω_0 , corresponding to the shortest relaxation time in the Rose-Zimm spectrum. From ω_0 coming from that change in $|G^*(\omega)|$, the persistence length, l_p , can be evaluated using the relationship $\omega_0 \approx$ $k_BT/8\eta_s l_p^3$, where η_s is the solvent viscosity [58]. Additionally, it is possible to estimate the mesh size of the network, ξ ; according to the flexible polymer theory, the relationship between the mesh size and de elastic modulus, G_0 , is $\xi^3 = (k_BT/G_0)$ [5,59]. Is important to note that the equation for calculating ξ is different from using for WLMs solutions; there, ξ considers the reptation of the

chain that is included in the factor
$$A; \xi \cong \left[A \cdot \left(\binom{k_B T}{G_0}\right)\right]^{\frac{1}{3}}$$
 with $A = 9.75$ [16].

4.2.3. Results and Discussion

Gelation temperature for physical gels. An example of how T_g is obtained for gelatin solution at C = 16 Wt % is presented in Fig. 4.11. For temperatures ≥ 40 °C, $G'(\omega)$ is proportional to ω^2 and $G''(\omega)$ to ω over a significant frequency domain, a behavior characteristic of polymer solutions [5]. As the temperature is lowered, both $G'(\omega)$ and $G''(\omega)$ get close and parallel, and eventually, when the temperature is equal to $T_g = 36.5 \ ^{\circ}C$, both moduli are almost the same in almost four orders of magnitude of the frequency. A *log-log* plot of $G'(\omega)$ or $G''(\omega)$ vs. ω forms straight lines with the same slope; the system has reached the critical gel. For $T < T_g$, the viscoelastic behavior is solid-like. Also, at the gel point, $tan(\delta)$ is independent of frequency, $tan(\delta)$ $= tan(n\pi/2)$, as seen in the inset of Fig. 4.11.b for 36.5 $^{\circ}C$ where the *n* value of Chambon & Winter criteria can be obtained, as well as the of stiffness, S. In this case, n = 0.62, equal to the results reported by Peyrelasse *et al.* [Peyrelasse] for a concentration range between 0.17-0.40 g/cm^3.



Figure 4.11. a) Linear viscoelastic spectra (SAOS protocol) at different temperatures for a gelatin solution (16 Wt %) to obtain a physical gel. b) $tan(\delta)$ vs. ω at different temperatures; insert $tan(\delta)$ as a function of the temperature for different frequencies. Image from Supplementary Information of [120].

Fig. 4.12 presents T_g values vs. C, between 3 Wt % and 16 Wt %. T_g increases as the concentration increases, *i.e.*, as more single chains are available, a higher temperature is needed for percolating. Along that concentration range, the average critical exponent is $n \sim 0.61$ (see inset of Fig. 4.12). Combining our data with that of Peyrelasse *et al.*[122], the power law behavior for gelatin critical physical gels is followed through a wide concentration range (3 Wt % to 40 Wt %). The inset of the inset of Fig. 4.12 also presents the stiffness factor as a function of gelatin concentration, which follows a power law, $S = 0.016C^{1.68}$, as in polymers [123].



Figure 4.12. T_g vs. gelatin concentration, the dotted line is a guide to the eye. Inset: *n* vs. *C*, and a log-log plot of *S* vs. *C*; the colored line is a linear fitting in the log-log plot ((regression coeficcient)² = 0.95). Image from [120].

Linear viscoelastic spectra of critical gels. A concentration of gelatin was chosen to study physical critical gels by mechanical rheology, and the gelation process was slow enough to have time for accurate measurements. Also, the gel must be strong enough to get reliable stress measurements. For C = 5 Wt %, the gelation time is ~ 90 min at $T_g = 30$ °C. For higher concentrations, gelation time decreases, making it challenging to obtain precise measurements. For C = 3 Wt % or below, gelation time increases; however, $G'(\omega)$ and $G''(\omega)$ show instabilities, possibly because the formed network is weak and prone to breaking for the imposed strains. Therefore, the concentration of gelatin solutions was C = 5 Wt % to reach the critical gel. Adding glutaraldehyde to gelatin solutions produces a chemical gel. Fig. 4.13 shows the G'(t) and G''(t) vs. t ($\omega = 0.5 s^{-1}$ and $\gamma = 0.8$ %) for different *R* values at 35 °C. Gelation time where $G'(t) \sim G''(t)$ decays as *R* increases (inset Fig. 13). For R < 0.15, gels are not formed because G'(t) always is below G''(t), but for R = 0.15, the gelation time is ~ 63 min. Therefore, we will use a relation of R = 0.20.



Figure 4.13. Isothermal time sweep curves for a gelatin solution under chemical gelation at C = 5 Wt % with different *R* values at 35 °C. Experiments were performed at $\omega = 0.5$ s⁻¹ and a strain of $\gamma = 0.8$ %. Insert: Gelation time as a function of *R*. Image from Supplementary Information of [120].

Fig. 4.14 presents the linear viscoelastic behavior for physical, chemical, and physicochemical critical gels, where $G'(\omega)$ and $G''(\omega)$ are parallel in several orders of magnitude in frequency. In Fig. 4.14, measurements were delayed after gelation started until the system was relatively close to the critical point to avoid observing a polymer solution in the first steps of gelation. In some figure panels, different vertical scales (y-axis) were used to see all measurements in one figure, regardless of the quench extent. In Fig. 4.14a shows $G'(\omega)$ and $G''(\omega)$ for different temperature quenches from an initial temperature equal to 35 °C to a final T_{f_r} (temperature quench with $\Delta T_q = T_f - 35 \ ^{\circ}C$). When the quench ends at $T_g = 30 \ ^{\circ}C$, $G'(\omega)$ and $G''(\omega)$ are parallel for more than three orders of magnitude in the frequency, *i.e.*, the solution became a critical gel for C = 5 Wt %; the exponent of the power law is $n = 0.64 \pm 0.03$. This figure also shows a quench below Tg~31 °C, where the systems are probably located in a post-critical gel state below the critical point. Here, two regions in the plots were found. In the first region, $\omega < 1 \text{ s}^{-1}$, the viscoelastic moduli have a slope > 1, decreasing as the temperature quench decreases. In a second region, ω $\geq 1 s^{-1}$, the slope is ~ 0.6; at short times (high frequencies), the system relaxes like a critical state. The origin of two slopes of the viscoelastic moduli is probably related to the structure change that occurs when the temperature is lowered from T_{g} , according to the work of Gho et al. [30]. Points measured faster, $\omega \ge 1 \ s^{-1}$, detect a different structure than those measured using longer times ω $\geq 1 s^{-1}$. As far as the temperature quench is close to T_g , the slope difference between these two

regions is smaller until $G'(\omega)$ and $G''(\omega)$ are parallel. Fig. 4.14b presents two critical chemical gels for R = 0.15 and R = 0.20, developed at 35 °C; these gels are established by covalent bonding [121]. In both cases, $G'(\omega)$ and $G''(\omega)$ are parallel along more than three orders of magnitude in ω ; the exponents of their power laws are $n = 0.79 \pm 0.02$ and $n = 0.70 \pm 0.01$ for R=0.15, and R = 0.20, respectively. In Fig. 4.14c, $G'(\omega)$ and $G''(\omega)$ are plotted for physicochemical gels with R = 0.15; gelation started when glutaraldehyde is added at 35 °C, and a temperature quench to a temperature T is applied immediately ($\Delta T_q = T - 35$ °C), *i.e.*, two gelation mechanisms run simultaneously. The critical gel is found at $\Delta T_q = -5$ °C, where moduli are parallel and follow a power law for more than three orders of magnitude in ω ; $n = 0.57 \pm 0.01$. However, in a post-critical gel state for quenches ending at $T < T_g$, *i.e.*, the gels inherit the behavior found in physical gels below the gelation temperature. They are parallel in a small range of ω ($\omega < 1 s^{-1}$) and follow a power law with a large exponent. After that, frequency moduli are parallel and have a power law close to that of the critical gel.



Figure 4.14. Linear viscoelastic spectra for physical, chemical, and physicochemical critical gels for gelatin solutions with C = 5 Wt %; $G'(\omega)$ and $G''(\omega)$ are plotted in different vertical scales in color. a) Physical gels for different quenches, $\Delta T_q = T$ − 35 °C: ΔT_q = −10 °C (black), $\Delta T_q = -8 \ ^{o}C$ (blue), $\Delta T_q = -6 \ ^{o}C$ (red), and $\Delta T_q = -5 \ ^oC$ (green). b) Critical chemical gels at 35 ^{o}C for R = 0.15 (black) and R =0.20 (blue). c) Physicochemical gels for R = 0.15. Gelation started when added the glutaraldehyde at 35 °C, and immediately a temperature quench was applied, $\Delta T_q = T - T_q$ 35 °C: $\Delta T_q = -8$ °C (black), $\Delta T_q =$ $-6 \ ^{o}C$ (blue), and $\Delta T_q = -5 \ ^{o}C$ (red). Image modify from [120].

Fractal dimension and strain hardening of mature gels. The strain-hardening behavior of different matured gels was studied using the LAOS protocol. According to Yang *et al.* [31], a matured gel is a gel where the viscoelastic moduli essentially do not vary with time. Fig. 4.15 shows the viscoelastic moduli for a physical gel as a function of time at a fixed frequency and strain ($\omega = 0.5 \ s^{-1}$ and $\gamma = 0.8 \ \%$), obtained at 29 °C. Here, G'(t) > G''(t) by around an order of magnitude; for

times greater than 240 min, the viscoelastic moduli essentially do not change. For this gel, the gelation time is around ~ 55 min at 29 °C, the largest gelation time of the studied systems. From a practical point of view, is enough consider a gel aged four hours to be a matured one, whether physical, chemical, or physicochemical.



Figure 4.15. Isothermal sweep curves for physical gelatin gels with 5 Wt % at T = 29 °C. Experiments were performed at $\omega = 0.5 \text{ s}^{-1}$ and strain of $\gamma = 0.8$ %. Image from Supplementary Information of [120].

Fig. 4.16 shows the viscoelastic moduli vs. shear strain ($\gamma = 0.1 - 5000$ %) for a fixed frequency ($\omega = 1 \ s^{-1}$) for different matured gels. $G'(\gamma)$ and $G''(\gamma)$ in matured gels exhibit similar trends, which could be described with three regions. In the first region, known as a Linear Viscoelastic Region (LVR), $G'(\gamma)$ and $G''(\gamma)$ are essentially independent of strain. In the second region, $G'(\gamma)$ and $G''(\gamma)$ increase with a positive curvature strain, and a sudden overshoot produces a third region where $G'(\gamma)$ and $G''(\gamma)$ decrease as the strain increases, suggesting a gelatin network failure where it starts to break. This failure can be easily seen in the $\sigma vs. \gamma$ curves presented in the insets of Fig. 3. As γ increases, $G''(\gamma) > G'(\gamma)$, which is related to the flow of a broken gel. The overshoot in the viscoelastic moduli is typical strain-hardening behavior for physical and chemical gelatin gels [31,124], which physicochemical gels inherit. According to the inserts of Fig. 4.16, the overshoot in the viscoelasticity moduli corresponds to a shear thickening effect because the stress increases with the strain before the breaking point.



Figure 4.16. Elastic and loss modulus for 5 *Wt* % matured gelatin gels *vs.* γ at a constant frequency ($\omega = 1 \ s^{-1}$). a) Matured physical gels at $T = 25 \ ^{\circ}C$, 27 $^{\circ}C$, and 29 $^{\circ}C$. b) Matured chemical gels at 35 $^{\circ}C$ for R = 0.15 and R = 0.20. c) Matured physicochemical gels with R = 0.15 at $T = 25 \ ^{\circ}C$, 27 $^{\circ}C$, 29 $^{\circ}C$, and 30 $^{\circ}C$. Insets, $\sigma vs.\gamma$, and the fittings to the BST model (continuous line). Image from [120].

According to discussed in section 3.3, to obtain information about the microstructure of the gel, was used the BST–scaling model to fit the nonlinear viscoelastic behavior of mature gels

to determine the fractal structure of the polymers in terms of the fractal dimension, d_f (see Eq. 3.15 and Eq. 3.16). The fittings of $\sigma vs.\gamma$ curves correspond to continuous lines in the insets of Fig. 4.16 (*(regression coefficient)² > 0.98* for all cases). Table 4.2 presents all parameters for the BST–scaling model and breaking strains, γ_{break} , corresponding to strain where the shear stress is maximum. For $\gamma > \gamma_{break}$, the stress decreases due to the broken gel flow.

	G ₀ (Pa)	n _{BST}	d_f	Y _{break} (%)						
Matured physical gels										
T = 25 °C	299.25 ± 5.08	3.36 ± 0.06	1.42 ± 0.01	200						
T = 27 °C	210.60 ± 0.61	3.07 ± 0.004	1.48 ± 0.001	267						
T = 29 °C	64.87 ± 11.45	3.18 ± 0.05	1.46 ± 0.001	297 ± 52						
Matured chemical gels										
R = 0.15	3.94 ± 0.99	2.62 ± 0.05	1.62 ± 0.02	861						
R = 0.20	55.07 ± 7.77	2.66 ± 0.04	1.60 ± 0.01	861						
Matured physicochemical gels										
T = 25 °C	464.00 ± 196.04	3.19 ± 0.16	1.46 ± 0.03	267						
T = 27 °C	327.34 ± 77.43	2.83 ± 0.08	1.55 ± 0.02	561 ± 115						
T = 29 °C	174.94 ± 5.84	2.73 ± 0.02	1.58 ± 0.01	643						
T = 30 °C	49.55 ± 12.20	2.74 ± 0.20	1.60 ± 0.04	480						

Table 4.2. Parameters associated with BST scaling model and break deformation of matured gels.

According to the master curve of Joly-Duhamel *et al.* [125], the concentration of the triple helices is proportional to $G_0 = \lim_{\gamma \to 0} G'(\gamma)$. From Table 4.2, for matured physical gels, G_0 decreases as the temperature where it was maturated increases, and so does the triple helices concentration, which agrees with previous reports [30]. However, γ_{break} slightly increases with temperature; a gel formed at 25 °C requires a lower strain to break the structure than a gel at 29 °C. One rupture mechanism unzips the triple helix junctions' zone [29–31]; the triple helices formed from the gelatin solution are shorter [30,126]when made at lower temperatures. On the other hand, G_0 increases with the relative glutaraldehyde concentration, R, for matured chemical gels, but it is quite lower than in physical gels, and γ_{break} is larger than in physical gels. G_0 for hybrid gels increases concerning physical gels at the same temperature; approximately, they are ~ 50 % larger from 25 °C to 27 °C and ~ 150 % at 29 °C. In this case, γ_{break} is higher than in physical gels due to the covalent crosslinking plus the physical crosslinking; however, γ_{break} is minor compared
to chemical gels. There is no clear trend with the temperature. Yan *et al.* [31] found a gel with C = 3 Wt % of gelatin and 0.2 Wt % of glutaraldehyde where the chemical networks were developed first, and after a temperature quench to allow physical networking, a so-called chemical-physical gel, the value of G_0 is additive ($G_0 = G_0^{physical} + G_0^{chemical}$). In physicochemical gels, the G_0 value is not additive. However, in promoting both routes of gelation at the same time, as presented here, G_0 increases more than in a chemical-physical gel, showing a technological advantage to promoting both gelation routes simultaneously.

For physicochemical gels, it depends on what crosslinking process dominates; at low temperatures, it is the physical crosslinking, and at higher temperatures, it is the chemical one. Nevertheless, the advance in one of them could modify the performance of the other: for instance, once a chemical bond occurs, the orientation of a section of the polymer could hamper a triple helix formation process. To support these statements, it is necessary to consider the following:

a) In the case of physical gelation, Chen *et al.* [127] estimated the temperature dependence of the sol-gel conversion rate constant. In the range of 30 - 19 °C, the rate of transformation increases as the temperature decreases, following the Arrhenius law, Ae^{-E_a/k_BT} , with $E_a = -130$ *kJ/mol*. For estimating the conversion rate above T_g for chemical gelation, the data given in Fig. 4.18a was used, which also follows an Arrhenius equation with $E_a = -92$ *kJ/mol*. Determining what process is going faster in the hybrid gel is more challenging as the temperature is ≤ 30 °C, because physical and chemical mechanisms work and interact simultaneously and cannot be separated to observe just one process alone. In both cases, the transformation rate increases as the temperature decreases. However, E_a is more negative in physical gels than in the chemical gel's E_a . Assuming that the E_a does not change too much below 30-31 °C, chemical bonding transformation is running slightly slower.

b) da Silva *et al.* [128] studied the gelation evolution in gelatins using the fraction of triple helices (χ) by optical rotation and G' at a fixed frequency ($\gamma = 1 \%$, $G' \sim G_0$) for the case of the physical, chemical, and hybrid gels. The last two employ glutaraldehyde as a crosslinker at different temperatures below T_g . In physical gelation, the lower the temperature, the higher χ and G'. For a chemical gel, there are no triple helices above T_g , and G' is several times lower than in the physical gel, and its time evolution during gelation is slower because as the crosslinker is consumed and the binding sites are occupied, the reaction will naturally slow down for hybrid gels. The value of G' increases as the temperature decreases, and they are larger than those of the physical gels at the same temperature. However, they reach saturation limits faster than in physical gels. χ are much lower than in physical gels at the same temperature, demonstrating that the formation of the chemical networks hinders the helical conformation change. There is a decoupling between χ and G'; not only triple-helix junctions contribute to G'. Increasing the temperature above T_g , the helix physical network is destroyed, leaving just the chemical scaffold with a G' much lower than in the physical gel. Also, G' was measured above T_{g} , and the same result was observed. Fig. 4.18c shows isothermal time sweep curves developed for a gelatin solution under hybrid gelation (5 Wt % and R = 0.15). The hybrid gel was formed at three different temperatures $\leq T_g$. Subsequently, the gel was melted to destroy the triple helices melted at 35 °C, allowing the remaining G' due to the chemical scaffold. As the temperature of hybrid gel formation is lower than T_g , $\Delta G'$ is larger, showing a greater quantity of triple helices than at temperatures closer to T_g . So, chemical bonding at low temperatures and a synergy between physical and chemical networks exists. The chemical networks obtained in the presence of the triple-helices present substantially higher G' values than those obtained just by gelating the sol phase.



Figure 4.18. a) Isothermal time sweep curves for a gelatin solution under chemical gelation at different temperatures with C = 5 Wt % and R = 0.15. Insert: Gelation time as a function of the temperature. b) Isothermal time sweep curves for a gelatin solution under hybrid gelation (5 Wt % and R = 0.15). The hybrid gel was formed at three different temperatures $\leq T_g$, and subsequently, the gel was melted to destroy the triple helices melted at 35 °C (indicated by an arrow), showing the remaining G' due to the chemical

scaffold; $\Delta G'(\%) = \frac{G'^{(formed at T < T_g)} - G'^{(melting gel at 35 °C)}}{G'^{(formed at T < T_g)}} \times 100$. Image from Supplementary Information of [120].

The self-similar network fractal dimensions [30,31,89,129,130] for the matured gels are displayed in Fig. 4.19. For physical gels, on average, $d_f \sim 1.45$, which is similar to that obtained by Yang et al.[31] using the LAOS for physical gelatin gels with C = 3 Wt % at 20 °C (d_f = 1.40 ± 0.05), and by Pezron *et al.* [131] using SANS with C = 5 Wt % ($d_f = 1.40 \pm 0.02$ for q = 0.20 - 1.66 nm⁻¹ with q the scattering vector). For chemical gels, we obtained $d_f \sim 1.61$ (average from R = 0.15 and R = 0.2), which is lower than $d_f = 1.48 \pm 0.02$ obtained by Yang *et al.* [31] These authors associated their value with the existence of rod-like structures, swollen coils, and small crosslinking aggregates within their network due to their high crosslinker concentration (~ 0.2 Wt % of glutaraldehyde in C = 3 Wt % of gelatin solution). In this case, the relative concentration of the crosslinker agent (~ 0.1 Wt % of glutaraldehyde in C = 5 Wt %) is lower than in Yang et al.[132] case. So, we expect that our network will have fewer crosslinking aggregates. The fractal dimension for chemical gels is close to $d_f \approx 1.7$, corresponding to the Flory swelling exponent v = 0.588 associated with isolated polymers in a good solvent [5,43]; these results suggest that the chemical gel networks are swollen interlocking Gaussian chains. For physicochemical gels, where two gelation processes run simultaneously, initiated at 35 °C, immediately guenched and matured to 25 °C, d_f is similar to that obtained for physical gels guenched and matured to 25 °C. For physicochemical gels initiated at 35 °C, immediately quenched and matured to 30 °C, d_f is similar to the chemical gels at 35 °C. However, physicochemical gels for other intermediate quenching temperatures have d_f values between 1.46 and 1.60. In Fig. 4.19, it seems as if the fractal dimension of hybrid gels, formed at 25 °C, corresponds to a gel whose network is mainly formed by a physical mechanism. In the same way, when the physicochemical gel is formed at 30 °C, the network formation is apparently dominated by a chemical mechanism producing a d_f similar to that of a chemical gel; the physical gelation is perturbed by the chemical crosslinking as discussed above. However, d_f apparently detects a mean of chemical and physical processes for the gelation process occurring at intermediate temperatures quenches. This would explain the trend we observe for d_f with temperature in Fig. 4.19. The conclusion is that the differences in mechanisms

to form the gel network can be slightly differentiated by the strain-hardening approach of matured gels, where the self-similarity of the network has a direct impact.



Figure 4.19. Fractal dimension for different matured gels aged over four hours. Image from [120].

LAOS and Lissajous-Bowditch curves. Figs. 4.20 and 4.21 present normalized 3D Lissajous-Bowditch curves (in red) and their projections for different matured gels in the elastic representation ($\sigma/\sigma_0 vs. \gamma/\gamma_0$ in green) and viscous representation ($\sigma/\sigma_0 vs. \dot{\gamma}/\dot{\gamma}_0$ in blue). They are measured at $\omega = 1 s^{-1}$, and σ_0 , γ_0 and $\dot{\gamma}_0$ are the maximum shear stress, shear strain, and shear rate in an oscillation cycle, respectively. Diagrams with a blue shadow indicate LB curves corresponding to an imposed strain deformation of $\gamma_0 = \gamma_{break}$. LB curves for strain cycles in the LVR zone are not presented because they are independent of strain; in this region, the loci of $\sigma/\sigma_0 vs.$ γ/γ_0 and $\sigma/\sigma_0 vs. \dot{\gamma}/\dot{\gamma}_0$ is a diagonal line or a circle, respectively, corresponding to solid-like behavior (see discussion of section 3.2).

For matured physical gels before $\gamma \le \gamma_{break}$, the nonlinear viscoelastic behavior is similar for temperatures 25 °C \le T \le 29 °C. For $\gamma > \gamma_{break}$, the LB curves are more similar for 25 °C and 27 °C. However, all of them differ for γ slightly larger than γ_{break} , although for 29 °C it is more notorious. Nevertheless, they all behave close to liquid-like when $\gamma \gg \gamma_{break}$. In LB curves (see Fig. 4.20), there are small oscillations (25 °C and 27 °C), which are not as strong as those observed by Goudoulas & Germann [124], probably because the gels are weak ($G \ll 3000 Pa$). Their gelatin gels have a $G \approx 3000 Pa$ (C = 3 Wt %, matured for 30 min and 90 min after a large quench ending to $T_f = 5 °C$). LB curves presented here are similar to recent simulation results for colloidal gels [85]. For matured chemical gels at $\gamma_0 = 110$ %, we observe a small deviation from the linear viscoelastic behavior. In the $\sigma/\sigma_0 vs. \dot{\gamma}/\dot{\gamma}_0$ plane, circles are slightly elongated, and as γ_0 increases, but without gel breaking ($\gamma_0 \leq \gamma_{break}$), the deviation of the linear viscoelastic behavior can be significant; circles become rhomboid-like. On the $\sigma/\sigma_0 vs. \gamma/\gamma_0$, found deformed lines, which, as γ increases, appear as lines with a shoulder. This behavior seems to be invariant with the chemical crosslinker concentration. However, as observed in their projections, when $\gamma_0 > \gamma_{break}$, LB curves show significant changes. For $\gamma_0 = 1540$ %, the projections on both planes are analogous to deformed ellipses with similar areas, which corresponds to $G'(\gamma) \sim G''(\gamma)$ of the strain sweep curves (see Fig. 4.16b). As strain is $\gamma_0 \gg \gamma_{break}$, the deformed ellipses change even more. In viscous representations, deformed ellipses are elongated, suggesting that the shear rate is close to being in phase with the stress, or equivalent, in an elastic representation, deformed ellipses change to distorted circles because the strain is close to being out of phase with stress; approaching to a liquid-like behavior. As maturation temperature increases, LB curves for matured physical and chemical gels behave similarly.

LB curves for matured physicochemical gels (see Fig. 4.21) with $\gamma_0 \le \gamma_{break}$ behave likewise when aged at 25 °C and 30 °C, although, at 27 °C, and 29 °C, they present slight differences. The deformed circles transform into deformed rhomboids, which increase as strain increases, quite visible in the viscous representation. On the elastic representation, the line becomes a shoulder. For $\gamma_0 > \gamma_{break}$ LB curves for physicochemical gels matured at 30 °C and are similar to those where the mechanism to form the network was chemical. On the contrary, LB curves for physicochemical gel matured at 25 °C are more similar to those for physical gel aged at 25 °C; here, the physical mechanism is dominant. Therefore, the features of LB curves and fractal dimension in hybrid gels depend on which gelation mechanism is dominant.



Figure 4.20. Normalized 3D Lissajous-Bowditch curves and their projections (elastic representation: $\sigma/\sigma_0 vs. \gamma/\gamma_0$ or viscous representation: $\sigma/\sigma_0 vs. \dot{\gamma}/\dot{\gamma}_0$) for matured physical gels for different γ_0 , with *C* = 5 Wt. %, aged 25, 27, and 29 °C on the left, and matured chemical gels aged at 35 °C on the right. Blue shadow corresponds to an imposed strain deformation $\gamma_0 = \gamma_{break}$. Image from [120].



Figure 4.21. Normalized 3D Lissajous-Bowditch curves and their projections (elastic representation: $\sigma/\sigma_0 vs. \gamma/\gamma_0$ or viscous representation: $\sigma/\sigma_0 vs. \dot{\gamma}/\dot{\gamma}_0$) for matured physicochemical gels for different γ_0 , aged at 25 °C, 27 °C, 29 °C, and 30 °C from a solution with C = 5 wt % and R = 0.15. Blue shadow corresponds to an imposed strain deformation $\gamma_0 = \gamma_{break}$. Image from [120].

Kinetic of gelation: Mean square displacements of microspheres embedded in gelatin solutions. The gelation kinetics process is followed by measuring the MSD of microspheres (500 nm) embedded in gelatin solutions using DWS, which in turn allows evaluating $G'(\omega)$ and $G''(\omega)$ according to described in Chapter 3 (see section 3.3). The MSDs of the microspheres depend on the viscoelastic environment, which, in our case, evolves from a sol to a gel state as time elapses. Although microspheres with different diameters (799 nm and 500 nm) were tested, the selected ones were less noisy at short times, and the MSD plateaus were more clearly defined.

Fig. 4.22 shows *MSD vs. t* curves for gelatin solutions at different times after a temperature quench to produce a physical gel ($T < T_g$), after adding glutaraldehyde for chemical gels ($T > T_g$), and simultaneously after adding glutaraldehyde and quenching ($T < T_g$) for physicochemical gels. The elapsed time since the quench or glutaraldehyde addition will be named t_q . For solutions that produce physical gels with C = 5 *Wt* %, particles move in a simple liquid (sol state) at $t_q = 10$ min after the quench started from 35 °C to 25 °C (see Fig. 4.22a). This behavior prevails for 20 min after the quench; a change onset occurs at $t_q \sim 75$ min. The *MSD vs. time* curves bend to form a shoulder, up to $t \sim 0.02$ s. The shoulder is more pronounced for $t_q > 75$ min; their height decreases as t_q increases. From a mesoscopic point of view, a shoulder in *MSD vs. time* curves is evidence of partial particle confinement. They explore all the available volume in the polymer network cage formed around it, limiting the displacement of the microsphere similar to those described by Sarmiento-Gomez *et al.* [133,134]. However, the cages formed by the polymer network slowly break and reform due to the physical nature of physical gel crosslinking. They are not permanent, allowing the particle to explore a larger space as time elapses; if particles were completely trapped, the curves would be flat horizontally.

Fig. 4.22b shows the MSD curves of colloidal particles embedded in gelatin solutions (C = 3 Wt % with R = 0.25), which form a chemical gel at 35 °C. For $t_q \le 30$ min, the MSD corresponds to simple diffusion at short times, $MSD \sim t$, although when they reach 10^{-4} s, the MSD vs. t curves start to bend. For $t_q > 30$ min, the MSD curves show a flat plateau, indicating that particles are confined. The flat shape of the plateau corresponds to particle movement in a polymer network cage that cannot relax due to the permanent chemical nature of the crosslinking. It is not possible to see the evolution of the MSD curves for the gelation process in less soft chemical gels (C > 3 Wt.

%) because the network completely traps them; correlation functions vs. time abruptly drop because particles are essentially fixed (not shown).

The MSDs for physicochemical gels have a mixed behavior between physical and chemical gels. At $t_q = 20$ min, the MSD vs. time curve has bent to form a shoulder, and at $t_q > 20$ min, the shoulder becomes increasingly horizontal, similar to the physical gel at the same temperature: 25 °C. In the case where the final quench temperature increases, the MSD vs. time curves become more horizontal as in a chemical gel, *i.e.*, more confinement (see Fig. 4.22d for T = 27 °C and Fig. Fig.4.22e for T = 30 °C); covalent junctions hinder triple helix formation. Consequently, the chemical bonding contribution increases the network cages' rigidity. The MSD vs. t curves for microspheres embedded in hybrid gels show a slight dependence on gelation mechanisms that is more obvious at early times, which agrees with the contribution of both mechanisms running simultaneously, but at very long times, particles are finally trapped.



Figure 4.22. MSD vs. time for microspheres embedded in gelatin solutions. a) Physical gel for C = 5 Wt % (dia. 500 nm and vol. fraction 0.025) after a temperature quench to 25 °C. b) Chemical gel for C = 3 Wt % (dia. 799 nm and vol. fraction 0.025) after adding glutaraldehyde (R = 0.25) at 35 °C. c) Physicochemical gel formation for C = 5 Wt % (dia. 500 nm and vol. fraction 0.025) after adding glutaraldehyde (R = 0.25) at 35 °C. c) Physicochemical gel formation for C = 5 Wt % (dia. 500 nm and vol. fraction 0.025) after adding glutaraldehyde (R = 0.15) and simultaneously quenching to 25 °C. d) Physicochemical gel formation for C = 5 Wt % with R = 0.15, and quenching to 27 °C. e) Physicochemical gel formation for C = 5 Wt % with R = 0.15, and quenching to 30 °C. Open circles correspond to experimental MSD data, and continuous lines correspond to best fitting using the Bellour et al. model [111]. Image modify from [120].

Viscoelastic spectra and mesoscopic lengths. To describe the temporary evolution of viscoelastic features of gelatin solutions from sol to gel were used the best fits to the Bellour et al. [111], model (see equation 3.70) for the MSD vs. t data (showing with a continuous line in Fig. 4.22). Contrary to WLMs solutions, $D_m = 0$ because particles are trapped in the network and cannot leave it.-Fig. 4.23a shows the time evolution of viscoelastic spectra of a gelatin solution after a temperature quench from 35 °C to 25 °C to produce a physical gel, measured from $t_q = 10 \text{ min}$ to $t_q \sim 240$ min, passing through the gel point. Fig. 4.23b-e shows a similar gelation process to obtain chemical and physicochemical gels. All gels, 10 min after starting the gelation process, behave as in a sol state $(G''(\omega) > G'(\omega))$, consistent with the simple diffusive dynamics of microspheres embedded in a liquid. As t_q increases, the gel reaches the gel point where $G'(\omega) \sim G''(\omega) \sim \omega^n$, in a range of frequencies from $\omega \sim 10^2 - 10^4 \, s^{-1}$, which is similar to the data of Cardinaux *et al.*[134] for C = 2 Wt % at 20 °C. For large elapsed times after the quench, all gelatin solutions reach the gel state $(G'(\omega) > G''(\omega))$, and the evolution of viscoelastic spectra is similar for all of them. The elapsed time for all solutions to reach the gel point after a quench was more extended in microrheology experiments than those developed in macroscopic rheometers because the number of crosslinkings needed by the network to percolate is larger in DWS cuvettes (~ 2.3 mL) than in the rheometer geometry (~ 1.2 mL); however, they have the same information, and viscoelastic spectra in the gel point are similar. It is impossible to discern which gelation process dominates in hybrid gels, which is consistent with our results of SAOS at gel points.



Figure 4.23. Time evolution of viscoelastic spectra for gelatin solution. a) Physical gel formed after a temperature quench from 35 °C to 25 °C at different t_q . b) Chemical gel formation for C = 3 Wt % at different t_q after adding glutaraldehyde (R = 0.25). Physicochemical gel formation for C = 5 Wt % at different t_q after adding glutaraldehyde (R = 0.15) and simultaneously quenching: c) 25 °C, d) 27 °C, and e) 30 °C. Image modify from [120].

An advantage of measuring gelatin flexible polymer solutions' viscoelastic spectra at high frequencies is treating them like other biopolymers [48,135] or living polymers [20,21,86,113]. In a semidilute regimen concentration (> 1 Wt % for gelatin chains [61]), polymer chains can have different stress relaxation modes. As explained, stress relaxes at high frequencies first dominated by the Rouse-Zimm modes and at even higher frequencies by the internal bending modes of Kuhn segments. The frequency for the change, ω_0 , allows us to estimate the persistence length in the polymer network. The inset of Fig. 4.24a presents $|G^*(\omega)|$ vs. ω for a gelatine solution quenched to 25 °C at $t_q = 40$ min. It shows the frequencies where $|G^*(\omega)| \sim \omega^{3/4}$, $|G^*(\omega)| \sim \omega^{5/9}$, as well as ω_0 . Fig.4.24a shows the frequencies where one of the relaxation modes dominates and ω_0 for different t_q up to 8 h after the gelation process started for producing a physical gel. In the same way, ω_0 is presented in Fig. 4.24b for a chemical gel and in Fig. 4.24c, for a physicochemical gel with two gelation mechanisms running simultaneously. The place where Rouse-Zimm modes dominate is observed in all solutions below ω_0 , which increases as t_q increases, while bending modes dominate at even higher frequencies until we cannot measure them. For the hybrid process, quenched to 30 °C bending modes persist until 8 h after the gelation process started (not shown), displaying an important difference from the other gels. It is important to note that microspheres embedded in the gel measure bending modes for $\omega < 10^6 \text{ s}^{-1}$, which is the limit of the DWS-microrheology technique [23,91,94,113].



Figure 4.24. Relaxation modes and $\omega_{0.}$ a) Frequencies where the relaxation modes dominate colored arrows, and ω_{0} (full circles) for different t_{q} up to 8 h after the gelation process has started to produce a physical gel C = 5 Wt %. Inset: Power-law exponents of $|G^*|$ Rouse-Zimm modes $|G^*(\omega)| \sim \omega^{3/4}$ in orange, and bending modes $|G^*(\omega)| \sim \omega^{5/9}$ in green, and ω_{0} (full red circle). (b) ω_{0} (full circles) for a chemical gel developed at 35 °C with R = 0.25and 3 Wt. %. (c) ω_{0} (full circles) for a physicochemical gel quenched to 27 °C with R =0.15 and 5 Wt %. Image from [120].

Fig. 4.25 presents the persistence length of polymer chains for gels with different gelation processes, *i.e.*, the length at which polymer chains appear straight in the presence of thermal undulations given by $l_p = \kappa/k_BT$, where κ is the chain bending modulus. In physical gelation (C = 5 Wt %), l_p starts at ~ 26 nm and decreases to $l_p ~ 10$ nm at $t_q ~ 190$ min. In chemical gelation (C = 3 wt % and R = 0.25), l_p starts at ~ 27 nm and decreases to ~ 12 nm at $t_q ~ 90$ min; l_p reaches a constant value at $t_q ~ 200$ min; apparently, this is the limiting of evolution for l_p in a network formed by covalent crosslinking. For the hybrid case, where two mechanisms are evolving simultaneously, l_p starts at ~ 24-28 nm depending on the glutaraldehyde concentration, and it decreases to $l_p ~ 10$ nm at $t_q ~ 360$ min for the gel quenched to 30 °C. This l_p is observed up to 8 h. The evolution of l_p as a function of t_q is slow despite two gelation mechanisms running simultaneously. For the physicochemical gel quenched to 25 °C, l_p decreases to 10 nm at $t_q ~ 75$ min, comparable to the physical process, which seems to be the dominant mechanism when the solution is quenched to this temperature. However, it is unclear why the speed at which l_p decreases to 10 nm is greater than in the other hybrid processes. For the hybrid process quenched to 27 °C, the evolution of l_p

has an intermediate behavior compared with the physical and hybrid process quenched to 30 °C. The crucial point for understanding Fig. 4.24a is what l_p refers to in these biopolymer chains. As mentioned, the network is made of segments with triple chains, which must be semiflexible with a large l_p as in other cases where $l_p \ge \xi$ [5], and of unstructured non-bonded flexible polymer sections. It is important to mention that turbidity in our gelatin is quite low, so we ruled out the formation of semiflexible collagen fibrils [136]. Then, l_{ρ} measurements capture the persistence length of the non-bonded flexible polymer sections where $l_p \leq \xi$. These polymer sections are relatively large when the gelation starts in all processes. As time elapses, the length of these polymer chains (Kuhn segments) appearing straight in the presence of thermal undulations decreases due to the formation of bonds. In this way, the curves for the gels undergoing physical and chemical processes seem similar. However, in the hybrid case, it is clear that these processes are not independent. As mentioned, covalent crosslinking hinders the formation of triple helices at $T \leq T_g$; the lower the temperature quench, the faster the decay of I_p , where physical gelation dominates. In quenches to 25 °C, Ip decays faster where the physical process dominates, and when the chemical process dominates, the decay is slower. At 27 $^{\circ}C$, we observe that I_p decays in an intermediate form between these two cases.

Fig. 4.24b presents the evolution of the gel network's volume mesh size, ξ , during the gelation process, which roughly follows an exponential decay. The general idea of mesh size is that a snapshot of a dense polymeric solution at a particular instant of time looks quite similar to an intricate network with a particular mesh size, independent of the presence of crosslinks. Consistent with what occurs in flexible polymers, ξ is much larger than I_p . Roughly, the mesh size in a physical gel is larger than in a chemical gel, but at long times, the last one is larger than in hybrid gels, where the lower the quench temperature, the smaller the mesh size. As in the case of I_p , for the hybrid gels, the decay of ξ as time elapses seems to have no relation with the pure physical or chemical processes, which seems to be another effect where the physical and chemical processes are not independent when running simultaneously.



Figure 4.24. Characteristic mesoscopic scales as time elapses after the gelation process has started for physical, chemical, and physicochemical gels. a) l_p vs. time; dashed lines are a guide to the eye. b) ξ vs. time; lines correspond to exponential decay fits. Image from [120].

4.2.3. Conclusions

The critical exponents for physical and hybrid critical gels are relatively close ($n \sim 0.6$), and for chemical gels, $n \sim 0.70 - 0.79$, slightly depending on the *R*-value. The features of LB curves and fractal dimensions in hybrid gels depend on the dominant gelation mechanism. Differences in forming the gel network were, to some extent, distinguished by the strain-hardening approach of matured gels, where the self-similarity of the network has a direct impact. Apparently, d_f results from a competition between physical and chemical gelation processes for physicochemical gels. For $\gamma > \gamma$ break, LB curves for physicochemical gels matured at 30 °C are similar to those where the mechanism to form the network was chemical. On the contrary, LB curves for physicochemical gel matured at 25 °C are more similar to those for physical gel aged at 25 °C; here, the physical mechanism is dominant. As gelation evolves, the MSD curves develop a shoulder in physical gels, which never becomes flat because the cage formed around microspheres can break and reform. Chemical gels completely trap microspheres due to the chemical nature of crosslinking, so the MSD vs. t curves form a plateau. The MSD vs. t curves for microspheres embedded in hybrid gels show a slight dependence on the gelation mechanism; at very long times, particles are trapped. At the gel point, it is impossible to discern which gelation process dominates with microrheology, in agreement with the SAOS results for critical gels.

As time elapses after gelation starts, the persistence length of the unstructured, nonbonded flexible polymer sections decreases due to the formation of bonds. The curves for the gels undergoing physical and chemical processes seem similar. However, in the hybrid case, it is not a simple mixture of both processes since they are not independent. As far as we know, this is the first time that the evolution of scales of the mesoscopic structure has been observed after the critical gel has been reached with different mechanisms running simultaneously. The time evolution of the gel network's mesh size roughly follows an exponential decay. The lower the temperature quench, the smaller the mesh size. Understanding the behavior of ξ could be relevant for diffusing proteins or nanoparticles in biopolymer networks with applications in different fields. For ξ , as for l_p , the decay as time elapses for hybrid gels seems to have no relation with the pure physical or chemical processes, which seems to be another effect where physical and chemical processes are not independent when running simultaneously.

4.3 Linear and nonlinear viscoelasticity of flexible charged biopolymer gels



GRAPHICAL ABSTRACT

The Chambon & Winter criteria indicate a similar critical exponent near the gel point, n \sim 0.6. This demonstrates that, regardless of the physical mechanism of gelation, the percolation threshold associated with network formation remains consistent.

4.3.1 Abstract

The linear and nonlinear viscoelastic behavior and their relationship with the mesoscopic structure of different physical gels made from polysaccharides (alginate, chitosan, and their mixture) were investigated. Various physical mechanisms form all gels: the alginate gel was formed by complexation between alginate chains and Ca²⁺, the chitosan gel was formed by modification of the hydrophobicity of the medium, and the alginate/chitosan gel was formed by slow electrostatic interaction between the chains trough polyelectrolyte complexes (PECs) formation.

The Chambon & Winter criteria show a similar critical exponent near the gel point for all gels $n \sim 0.6$, demonstrating that, regardless of the physical mechanism of gelation, the percolation threshold associated with gel network formation is the same. In the previous section, a similar critical exponent for physical gelatin gels supported our observation of the similarity of critical physical polymeric gels. Also, the strain-hardening behavior of the matured gels was investigated using the LAOS protocol; the BST scaling model was used to interpret the results in mesoscopic

terms, such as the fractal dimension. For alginate gels, $d_f \sim 1.25$ -1.33, and its value depends on Ca²⁺ concentration; as Ca²⁺ concentration increases, the fractal dimension decreases, showing a difference in their network structure due to the formation of different forms of complexation between Ca²⁺ and alginate chains. For chitosan gels, $d_f \sim 1.33$ -1.49, and their value depends on the rate at which the modification of the hydrophobicity of the medium; at a high rate, d_f decreases. The alginate/chitosan mixture forms a microgel, confirmed by the nonlinear viscoelasticity spectrum and micrographs obtained by AFM. Fractal dimensions could not be determined for alginate/chitosan gels due to the limitations of the BST-scaling model. The Lissajous-Bowditch curves exhibit variations among all gels in the region of nonlinear viscoelasticity, serving like a fingerprint.

Additionally, diffusion wave spectroscopy micro-rheology was employed to measure the mean square displacements (MSD) of the microspheres embedded in the precursor solution to study the kinetics of gelation. In a kinetic study, we observed that the MSD decreased according to the formation of the network, and the shape of MSD is different according to the physical gelation mechanism. The MSD data can be used to obtain the viscoelastic spectra at high frequencies, allowing us to observe the different relaxation modes of the chains in the network. The results in this section correspond to the last part of this Ph.D. project and are presented as a preliminary discussion of the results. Discussions and writing of two papers are underway.

4.2.2. Experimental section

Materials. The alginic acid sodium salt of medium viscosity from brown algae (Mol. Wt. 324,138 Da, mannuronic acid to guluronic acid ratio (M/G) \approx 1.56, Sigma-Aldrich USA). Chitosan of high molecular weight (*Mol. Wt. 966,830 Da*, degree of deacetylation (DD) 74.8 %, Sigma-Aldrich USA), calcium carbonate (CaCO₃, purity > 99.0 %, Sigma-Aldrich USA), D-(+)-Gluconic acid δ -lactone (GDL, purity > 99.0 5, Sigma-Aldrich USA), acetic acid (HAc, purity > 99.8 %, Sigma-Aldrich USA), 1,2-propanediol (purity > 99 %, Germany), hydrochloric acid (37 Wt %, HCl, Sigma-Aldrich USA), sodium hydroxide (NaOH, purity > 99 %, Sigma-Aldrich USA), and sodium chloride (NaCl, purity > 99.0 %, Sigma-Aldrich USA). Solutions were prepared with ultrapure deionized water (Nanopure, USA). Except for chitosan, all chemical products were used as received. The moisture

of alginate and chitosan powered were determinate by thermogravimetric analysis (TGA, TA Instruments, USA): moisture of alginate and chitosan were *10.44 %* and *9.11 %*, respectively.

For chitosan purification, chitosan was dissolved at 1 w/v % in acetic acid at 0.5 Wt % under magnetic stirring at ~ $30 \degree C$ overnight. After complete dissolution, it was centrifuged at ~ 4500 rpm by 2 h, and then the immiscible impurities were decanted. The solution without visible impurities was filtered successively on 20 and 8 μm membranes (Millipore, USA). Then, the solution was precipitated with [NaOH] = 1 M up to a constant pH of ~ 11 and centrifuged at ~ 4500 rpm by 2 h. The precipitate was repeatedly washed with deionized water and centrifuged until a pH of ~ 7 (on average four times). Finally, the precipitate was dry at room temperature in a chamber vacuum. The moisture was determined by thermogravimetry analysis; on average, moisture was 6.4 Wt %.

The molecular weight of alginate and chitosan was determined using the Mark-Houwink-Sakurada (MHS) equation, $[\eta] = KM_W^{\alpha}$; here, $[\eta]$ is the relative viscosity ($[\eta] = \lim_{c \to \infty} \frac{\eta - \eta_s}{c \eta_s}$, η the viscosity of the polymer solution, η_s the viscosity of the solvent, and c the polymer concentration, mg/mL), M_w is the molecular weight, K and α are constants related to the interaction of the polymer with the medium [137–140]. Figure 4.25a shows the determination of relative viscosity by extrapolation of viscosity data; the continuous lines corresponds to linear fit: $y = 1074 + 91 \times 10^4$ x with (regression coefficient)² = 0.94 for alginate solutions, and y = $667 + 90 \times 10^{4}$ x with (regression $coefficient)^2 = 0.98$ for chitosan solutions. The constants of the MHS equation are K = $0.0073 \ mL/g$ and $\alpha = 0.92$ for alginate solution with [NaCl] = 0.1 M at 25 °C [139], and K =0.00181 mL/g and $\alpha = 0.93$ for chitosan solution solutions in an acid medium ([HAc] = 0.1 M) at 25 °C [137,138]. Fig. 4.25b shows the linear potentiometric titration for a chitosan sample. The procedure was: 0.1201 g of chitosan (W) was dissolved into 35 mL (V_A) of [HCl] = 0.8573 M (C_A); the solution was titrated potentiometrically with [NaOH] = 0.517 M (C_B) by steps of 1 mL under magnetic stirring; the titration curve having two inflection points (see Fig. 4.25b) where the first (1) and second (2) inflection points are the equivalence points of the titration of excessive HCl and protonated chitosan, respectively. Due to the precipitation of chitosan in the neutral pH range, the second inflection point does not coincide with the actual equivalence point. To calculate the volume of NaOH for the titration of protonated chitosan (V_e), Ingman & Still [141] proposed a linear relationship for the titration curve, $V_{NaOH} = V_e - \left(\frac{V_A + V_{NaOH}}{c_B}\right) \left([H^+] - [OH^-]\right)$ (see insert of Fig. 4.25b; the continuous line correspond to linear fit y = 57 + 1.54x with (regression coefficient)² = 0.98). Finally, DD (%) is calculated from the relationship proposed by Tan *et al.*[142];



Figure 4.25. a) $\frac{\eta - \eta_s}{\eta_s c}$ *vs. c* for alginate solutions with [NaCl] = 0.1 M at 25 °C, and chitosan solutions in an acid medium ([HAc] = 0.1 M) at 25 °C. b) *pH vs. V_{NaOH}* of NaOH added to chitosan in an acid medium. Inset: V_{NaOH} *vs.* $-\left(\frac{V_A+V_{NaOH}}{c_B}\right)([H^+] - [OH^-])$.

Gelation protocol. This work investigated the differences in the linear and nonlinear viscoelastic behavior of three physical gels made from polysaccharides: alginate, chitosan, and the mixture alginate/chitosan (Alg/Chit), where each gel is formed following a different formation protocol. The following presents some general characteristics of polysaccharides and an outline of their gel formation protocol.

i) Alginate gel

Alginate is an anionic linear copolymer of $(1 \rightarrow 4)$ -linked β -L-mannuronic (M) and α -L-guluronic (G) residues. The residues are arranged randomly with GG, MM, and GM-dimer blocks, interspersed along the polymer chain, as shown in Fig. 4.26a. Sodium alginate can be ionically cross-linked by divalent ions [143,144]. The stereochemistry of the G monomer allows for the formation of a remarkably stable cross-link structure called an "egg box." In the simple egg-box structure, two alginate chains are connected by a divalent ion by coordinating four carboxylic groups. The links are mostly favored among G blocks of more than eight units (rod-like junctions) and can even give rise to bundles of junctions with more than two chains involved. Even if the egg box determines the formation of a gel, it is worth noticing that other ionic cross-links, *e.g.*, between M-M and M-G units [143]. Fig. 4.26a shows a cartoon of the alginate gel, such as the egg-box structure.

The gel formation protocol was as: Alginate was dissolved in deionized water under magnetic stirring at approximately 30°C overnight. To form a gel, CaCO₃ was dispersed in an alginate solution under vigorous vortex stirring for ~ 1 min, and subsequently, GDL was added to the mixture of alginate/CaCO3 under vortex stirring, promoting the gelation mechanism. The alginate concentration was fixed ($C_{alginate} = 1.8 \text{ w/v} \%$), varying the quantity of CaCO₃ and GDL ([$CaCO_3$] = 0.3, 0.4 and 0.7 mM, $C_{GDL} = 6-18 \text{ mg/mL}$). GDL is a slowed-down acidification substance promoting the solubilization of CaCO₃, $CaCO_{3(s)} \rightleftharpoons Ca^{2+} + CO_3^{-2}$ with a solubility constant of $K_s = 10^{-6.05}$ [145], trough carbonic acid (H₂CO₃) formation, which chemical equilibrium is $H_2CO_3 \leftrightarrows 2H^+ + CO_3^{-2}$. In this way, it was possible to obtain a Ca²⁺ slowly and thus study a gel formation from sol to gel contrary to alginate gels formed with CaCl₂; using CaCl₂, the gelation is quick due to instantaneous solubilization of the calcium source [37,146].

ii) Chitosan gel

Chitosan is a polysaccharide produced from the N-deacetylation of chitin under alkaline conditions [137]. Chitin is a mucopolysaccharide abundant in nature and the support material of crustaceans, insects, etc. [137], and is a linear polymer $(1\rightarrow 4)$ -2-acetamido-2-deoxy- β -D-glucan (GlcNAc). The N-deacetylated derivative of chitin is never complete. Therefore, chitosan belongs to the family of linear copolymers of GlcNAc and $(1\rightarrow 4)$ -2-amino-2-deoxy- β -D-glucan (GlcN). The degree of acetylation (DA) is an important parameter, corresponding to the molar fraction of acetyl units constituting the polymeric chains. For DA lower than 60 %, chitosan is solubilized in dilute acid solutions. One way to form a chitosan gel is by modification of the hydrophobicity of the medium. Domard [147,148] formed a gel using a mixture of chitosan dissolved in an acid medium and 1,2-propanediol in an equivalent amount. They showed that gelation is achieved for a critical value of the balance between hydrophilic and hydrophobic interactions; here, the gelation mechanism is attributed to the formation of physical junctions due to hydrophobic

interactions involving N-acetyl groups and hydrogen bonding. Fig. 4.26b shows a cartoon of the chitosan gel.

The gel formation procedure followed the chitosan purification protocol; before precipitating the chitosan with NaOH, the chitosan solution was mixed with 1,2 propanediol in a 50:50 ratio under magnetic stirring at room temperature for ~ 30 min. The solution was centrifuged for 30 min at ~ 4500 rpm to remove bubbles. The concentration of chitosan and HAc was adjusted to obtain a chitosan solution at 0.9 w/v % in HAc 0.5 Wt %:1.2 propanediol. The gelation process starts by drying the chitosan solution; the water evaporates first, then the alcohol, changing the dielectric constant of the solution. Thus, the medium becomes more hydrophobic with time.

iii) Alginate/chitosan gel

Chitosan dissolves in an acidic medium because the amino group accepts a proton from the medium $(-NH_2 + H^+ \rightarrow -NH_3^+)$, charging the chain positively. If the pH is near and above the chitosan pKa = 6.3 (minus the logarithm of acidity constant), the amino groups dissociate, which leads to the polysaccharide precipitation; so, the solubility and charging of the chitosan chain depend on pH, so, it must take into account for a gelation method. The gelation of charged polymers lies in forming polyelectrolyte complexes (PECs) by electrostatic interaction between cationic and anionic counterparts as they are mixed together. However, an important limitation of chitosan is the precipitation of PECs, usually caused by the strong electrostatic interaction of the chains, which can be seen as phase separation; one phase rich in polymer and another poor in polymer, usually called coacervate. Fig. 4.26e shows a coacervate formed by fast acidification using a strong acid (HCl). Shchipunov et al. [149] proposed a method for in situ formation of chitosan hydrogel with anionic polysaccharides with chitosan. They proposed slowed-down acidification of solutions performed by GDL, gradually charging a chitosan chain (positive charges) and then smoothly increasing the attractive electrostatic interactions with anionic polysaccharides. Their experiments were performed to test gel formation by the gradual acidification of dispersed chitosan particles with alginate, carboxymethylcellulose, carrageenan,

sodium hyaluronate, and xanthan. The gelation protocol in this work is similar to that proposed by Shchipunov *et al.* [149]. The Fig. 4.26e shows the gel obtained with this method.

The formation gel procedure followed: First, alginate was dissolved in deionized water under magnetic stirring at approximately $30^{\circ}C$ by ~ 8 h at a fixed concentration ($C_{alginate} = 0.9 \text{ w/v}$ %). Subsequently, the chitosan was dispersed into an alginate solution at ~ 30 C overnight at different concentrations. The pH of chitosan dispersed in alginate solution was ~ 7.6 (> pK_a) and was adjusted at pH ~ 7 using [HCI] = 0.1 M; the HCl solution was dripped slowly into the solution alginate/chitosan under magnetic stirring at room temperature. Finally, GDL was added to the Alg/Chit solution, promoting the gel formation. Depending on the experiment, the chitosan concentration was varied ($C_{alginate} = 0.375 \cdot 1.125$ %), as well as that of GDL ($C_{GDL} = 6 \cdot 18 \text{ g/mL}$). In an approximate way, the number of charges in the chitosan and alginate chain was calculated based on the following argument:

- The number of charges in the alginate chain was calculated by considering the molecular weight of the alginate chain and the molecular weight of the M and G monomers that constitute the chain; one M and G monomers contribute a unity of charge, *-e.* Similarly, the number of positive charges in the chitosan chain was calculated considering the chitosan's molecular weight with a DD = 74.8 %, which indicates that the chain is charged 74.8 % due to the presence of GlcN groups. Here, one GlcN monomer contributes a unity of positive charge, *+e.* Table 3 shows the number of charges per unit of volume for different polymer concentrations, such as the ratio between positive and negative charges.

C _{polymer} (v/w %)	Number of charges/ N_A	Number of - e / Number of + e (N_e -/ N_e +)
Alginate at 0.9 v/w %	5.1×10^{-5}	
Chitosan at 0.375 v/w %	1.7×10^{-5}	~3
Chitosan at 0.75 v/w %	3.5×10^{-5}	~1.5
Chitosan at 1.125 v/w %	5.2×10^{-5}	~1

 Table 3. Number of charges per unit of volume for all polymer concentrations used to form Alg/Chit gel.

N_A corresponds to Avogadro's number ($N_A \approx 1.062 \times 10^{23}$).



Figure 4.26. Cartoon for different gelation mechanisms. a) Structure of alginate chain and formation of eggbox structure in the presence of Ca²⁺. b) Structure of chitosan chain and the hydrophobic junctions between chitosan chains. c) Alginate and chitosan chains and PEC formation through electrostatic interaction; coacervate formation by rapid acidification, and gel formation by low acidification. The length of chitosan and alginate chains are consistent according to the ratio between their molecular weight $(M_w^{(chitosan)}/M_w^{(alginate)} \approx 3)$.

Rheological measurements. All measurements for SAOS and LAOS were performed with an MCR-702 Twin Drive rheometer (Anton Paar, Austria) using a cone-plate geometry (2° and 4° cone angle, Diam. = 40 mm) and temperature control ($\pm 0.1 \ ^\circ$ C). A solvent trap was used to avoid water evaporation. Viscoelastic spectra close to the gel point. An estimation of the gelation time is given by the time needed to reach the gel point, *i.e.*, the elapsed time to reach $G'(t) \sim G''(t)$ along an isothermal time sweep experiment with a constant frequency ($\omega = 0.5 \text{ s}^{-1}$) and constant strain ($\gamma = 0.15 \%$ or 0.8 %). The viscoelastic spectra are performed after the gelation process starts. The measurement of $G'(\omega)$ and $G''(\omega)$ started after a specific elapsed time, close to the gelation time. All measurements were made at 25 °C, except for one chitosan gel formed at 20 °C.

Linear and nonlinear viscoelastic spectra of mature gels. The viscoelastic spectra were obtained on matured gels after 5 – 6 h of starting the gelation process. It was considered a matured gel when the viscoelastic moduli did not change with the time for 30 min in an isothermal time sweep experiment ($\omega = 0.5 \text{ s}^{-1}$ and $\gamma = 0.15$ or 0.8 % were fixed). Frequency sweep measurements were carried out in the range of $\omega = 0.1-100 \text{ s}^{-1}$ in the LVR ($\gamma = 0.8$ % for alginate and chitosan gels and $\gamma = 0.15$ % for Alg/Chit gels). Afterward, for LAOS measurements, strain-sweep measurements were developed ($\gamma = 0.1-5000$ % at $\omega = 1 \text{ s}^{-1}$). Lissajous-Bowditch curves were obtained for one imposed intra-cycle shear strain.

Microrheology and diffusive wave spectroscopy. The mean square displacement is measured with DWS, as described in section 3.3. Polystyrene microspheres (dia. 784 nm; Bangs Labs, USA) were added to the solutions (vol. fraction ~ 0.025), mixed, and placed in a rectangular optical glass cuvette (2 mm optical-path length, Sterna Cell, Inc.) at 25 °C. According to the last section, the gelation process of alginate and Alg/Chit starts when GDL is added to the polymer solution and mixed, and the gelation of chitosan starts when the cuvette is opened for the drying process; this time corresponds to the starting point of the gelation process for measuring the MSDs. The MSDs of probe microspheres are determined by collecting their scattered light from a speckle over 180 - 300 s, and the intensity auto-correlation functions are evaluated; measurements were taken along ~ 8 h for different waiting times after gelation started. Scattered light collected for 180 s is enough to consider local thermodynamic equilibrium with good statistics. From the MSD, the viscoelastic spectra ($\omega \approx 10^2 - 10^{\Lambda 6} \text{ s}^{-1}$) can be evaluated up to high frequencies; the experimental MSD curves for sol state (at times close to starting gelatin process) were fitted using a power law with two terms ($\langle \Delta r^2(t) \rangle = A_1 t^{\alpha_1} + A_2 t^{\alpha_2}$, A_1 , A_2 , α_1 , and α_1 are constants from fitting).

4.2.3. Results and Discussion

Linear viscoelastic spectra of critical gels. For the study of critical alginate gels by mechanical rheology, GDL concentration was chosen, and the gelation process was slow enough to have time for accurate measurements. In an isothermal time sweep experiment (at ω and γ fixed), the evolution of G'(t) and G''(t) is similar to that obtained for gelatin gels (see Fig. 4.13). For 6 mg/mL, 12 mg/mL, and 18 mg/mL, the gelation time (where G'(t) and G''(t) are crossing) are t_q ~143 min, $t_g \sim 59$ min, and $t_g \sim 41$ min, respectively. So, the concentration of GDL for the study of critical gels is 6 mg/mL. Figure 4.27 shows the viscoelasticity moduli close to the gel point; here, measurements were delayed after the onset of gelation until the system was close to the critical point to avoid observing a sol state. The time delayed before started the measurements were similar to gelation times obtained by an isothermal time sweep experiment: $t_g \sim 143 \text{ min}$, $t_g \sim 88$ min and $t_g \sim 98$ min for [CaCO₃] = 3 mM, [CaCO₃] = 5 mM and [CaCO₃] = 7 mM, respectively. For all panels of Fig. 4.27, different vertical scales (y-axis) were used to view all measurements in a single figure; the y-axis corresponding to the viscoelasticity moduli data are in the same color. For alginate critical gels, $G'(\omega)$ and $G''(\omega)$ are parallel along more than three orders of magnitude in frequency, and according to Chambon & Winter criteria [28], $G'(\omega) \sim G''(\omega) \sim \omega^n$ with n = 0.60 - 1000.65; according to Ca²⁺ increases the critical exponent decrease. The critical exponents values are good agreement with Liu *et al.* work [150]. They reported n = 0.620 for alginate gels induced by calcium cations through in situ release using GDL at Calginate = 3 Wt %; here, the polymer used is of low molecular weight with M/G = 1.85 close to M/G used in this work ($M/G \approx 1.56$).

The critical exponent reflects the nature of the size distribution of the mesoscale superstructure in the gel point [26,28,120]; for the case shown here, the *n* value depends slightly on the concentration of Ca²⁺, essentially. Compared to other works, the critical exponent for alginate gels depends strongly on the nature of the alginate chain, *e.g.*, molecular weight and mannuronic acid to guluronic acid ratio. For example, Lu *et al.* [146] reported different critical exponents for alginate gels at low and high molecular weights of polymer with $C_{alginate} = 2-6$ Wt %, using Ca-EDTA and GDL for promoting the gelation process. In their work, for alginate with low molecular weight with M/G = 0.6, the critical exponent is \approx 0.71 and independent of $C_{alginate}$, while



for high molecular weight alginate M/G = 91 with, *n* decreases from 0.72 - 0.37 with increasing $C_{alginate}$.

Figure 4.27. Linear viscoelastic spectra close to gel point for different physical gels: a) Alginate gels at 9 w/v %, 6 mg/mL GDL and CaCO3, b) chitosan gels at 0.9 w/v % in HAc 0.5 Wt %:1.2 propanediol drying at different temperatures, and c) alginate/chitosan gels at 0.9 w/v % of alginate with different quantities of GDL and chitosan. Different vertical scales (y-axis) are used where the color in y-axis corresponds to measuring data.

Figure 4.27b shows the viscoelasticity moduli for critical chitosan gels; here, the gelation process occurred when the solution of chitosan in HAc:1,2 propanediol is drying; during

rheological measurements, the samples were placed in a temperature control chamber and without evaporation trap. For gel dried at 20 °C and 25 °C, the critical exponent is n = 0.58 and n = 0.62, respectively; drying the solution of chitosan at 20 °C, $G'(\omega)$ and $G''(\omega)$ are parallel in three orders of magnitude in ω , while drying at 25 °C, $G'(\omega)$ and $G''(\omega)$ are parallel over four orders of magnitude in ω . The n values reported here are closed for thermosensitive chitosan- β -glycerophosphate at 35 °C ($M_W \approx 850,000$ Da, DD = 93 %), n = 0.61 [151]. However, as well as with alginate critical gels, there is a slight difference concerning chitosan gels with different molecular weights and deacetylation degrees. For example, Boucard *et al.* [148] reported a critical exponent n = 0.47 for chitosan gel at $C_{chitosan} = 3$ Wt % in HAc:1,2 propanediol dry at 50 °C ($M_W \approx 540,000$ Da, DD = 97.4 %), and Montembault *et al.* [147] reported the n = 0.5 for chitosan gels with $C_{chitosan} = 0.5-3$ Wt % formed by the neutralization of chitosan positively chains with ammonia ($M_W \approx 500,000$ Da, DD = 48-95 %). In summary, the critical exponents for alginate and chitosan critical gels have a good agreement with systems similar in molecular weight, and the M/G ratio for alginate gels validates the procedure shown here to obtain the critical exponent through rheological measurements.

Linear viscoelasticity spectra for Alg/Chit gels close to the gel point are shown in Fig. 4.27c. The chitosan concentrations were chosen to obtain three different ratios between the charges in the polymer chains: excess negative charge $(N_e - /N_e^+ \approx 1.5 \text{ and } \approx 3)$, and stoichiometric equivalency between charges $(N_e - /N_e^+ \approx 1)$. In early experimental exploration, isothermal time sweep experiments show long gelation times using $C_{GDL} = 18 \text{ mg/mL}$. However, using these delay times before starting the viscoelastic spectra measurement, the viscoelasticity spectra correspond to solid-like behavior $(G'(\omega) > G''(\omega))$. This underestimation of delay-time by the isothermal time sweep experiment may be because the perturbation ($\gamma = 0.15\%$ and $\omega = 0.55^{-1}$) can be enough to delay the formation of the infinity network. It was possible to obtain $G'(\omega)$ and $G''(\omega)$ parallel along more than three orders of magnitude in ω for higher $N_e - /N_e +$ adjusting the delay-time; here, n = 0.58. For lower $N_e - /N_e +$ ratios, it was not possible to obtain the viscoelasticity spectra close to the gel point. Lowering C_{GDL} to 6 mg/mL, the critical exponent for $N_e - /N_e + \approx 1.5$ increases to 0.64. For $N_e - /N_e + \approx 3$, $G'(\omega)$ and $G''(\omega)$ are quasi parallel with the frequency in fourth orders of magnitude in ω , showing a shoulder at low frequency; the system is in a post-gel state, where the transition between sol to gel state is faster than time need to acquire rheological measurements at low frequencies. However, given the *n* values for lower $C_{chitosan}$, the critical exponent for a gel with stoichiometric equivalency charges is expected to be $n \sim 0.6$. The critical exponents for Alg/Chit gels have not been previously reported.

In summary, the Chambon & Winter criteria show a similar critical exponent near the gel point for all gels n ~ 0.6, demonstrating that, regardless of the physical mechanism of gelation, the percolation threshold associated with gel network formation is the same. As previously mentioned, the *n* value depends on the nature of the polymer chain, and these values may vary slightly according to the measurement protocol as shown as demonstrated by Lu *et al.* [146] for alginate gels, as well as the dispersion of critical exponents for different polymer gels [27]. The results presented here show that the measurement protocol is the same for all critical gels, supporting the idea of the similarity of the polymeric network close to the gel point. Also, in the previous section, a similar critical exponent for physical gelatin gels was found to support the idea of the similarity of the physical gelatin gels was found to support the idea of the similarity of biopolymers' critical gels with a physical gelation mechanism.

Nonlinear viscoelasticity spectra of mature gels. Fig. 4.29 shows the viscoelastic moduli vs. γ for different matured gels in a wide range of strain, 0.1 - 5000 %, for a fixed frequency ($\omega = 1s^{-1}$); measurements started 5 – 6 h after starting the gelation process. The linear viscoelasticity spectra show a solid-like behavior ($G'(\omega) > G''(\omega)$) for all matured gels in a wide range of frequency, $\omega = 0.1-100 \ s^{-1}$ (not shown). $G'(\gamma)$ and $G''(\gamma)$ in matured alginate and chitosan (see Fig. 4.29a-b) gels exhibit similar trends, which could be described with three regions as well as gelatin matured gels. In the LVR region, $G'(\gamma)$ and $G''(\gamma)$ are constant. In the second region, $G'(\gamma)$ and $G''(\gamma)$ and $G''(\gamma)$ decrease as the strain increases, suggesting network failure where it starts to break. As γ increases, $G''(\gamma) > G'(\gamma)$, which is related to the flow of a broken gel. The overshoot in $G'(\gamma)$ and $G''(\gamma)$ can be easily seen in the σ vs. γ curves in the insets of Fig. 4.29 show shear thickening.





Figure 4.29. $G'(\gamma)$ and $G''(\gamma)$ vs. γ at a constant frequency ($\omega = 1 \ s^{-1}$) for matured gels. a) Alginate gels at 9 w/v %, 6 mg/mL GDL and CaCO3. b) Chitosan gels at 0.9 w/v % in HAc 0.5 Wt %:1.2 propanediol drying at different temperatures. c) Alg/Chit gels at 0.9 w/v % + 18 mg/mL GDL with different $C_{chitosan}$. d) Alg/Chit gels at 0.9 w/v % with different $C_{chitosan}$ and C_{GDL} . e) Photographers of Alg/Chit gels before of LAOS measurements. Insets, σ vs. γ , and the fittings to BST model (continuous line). The arrows indicate the yield point.

As well as gelatin matured gels, to obtain information about the microstructure of the gel network, was used the BST–scaling model to fit the nonlinear viscoelastic behavior to determine the fractal structure in terms of the fractal dimension (see Eq. 3.15). The fittings of σ vs. γ curves correspond to continuous lines in the insets of Fig. 4.29a-b (*(regression coefficient)*² > 0.98 for all cases). Table 4.3 presents all parameters for the BST–-scaling model and strain breaking.

	G ₀ (Pa)		n _{BST}		d _f		γ _{break} (%)		
Alginate matured gels: 1.8 w/v % + 6 mg/mL GDL with CaCO ₃									
[CaCO ₃] = 3 mM	12.8 Pa		4.02		1.33		199 %		
[CaCO₃] = 5 mM	58.1 Pa		4.64		1.28		111 %		
[CaCO ₃] = 7 mM	119.5 Pa		4.94		1.25		83 %		
Chitosan matured gels: 0.9 w/v % in HAc 0.5 Wt %:1.2 propanediol drying at different temperatures									
20 °C	16.59 Pa		3.05		1.49		271		
25 °C	43.55 Pa		4.00		1.33		202		
Alg/Chit matured gels: 0.9 w/v % of alginate with different quantities of GDL and chitosan.									
(G ₀ (Pa)	-	$\gamma_{1^{\circ}Y.P.}$ (%)		$\gamma_{2^{\circ}Y.P.}$ (%)			
$C_{chitosan} = 0.37, C_{GDL} = 18 \text{ mg/mL}$			30.3 Pa		0.78 %				
$C_{chitosan} = 0.75, C_{GDL} = 18 \text{ mg/mL}$		1	1244.3 Pa		2.48 %		111 %		
$C_{chitosan}$ = 1.12, C_{GDL} = 18 mg/mL		3	3496.5 Pa		3.33 %		267 %		
$C_{chitosan} = 0.75, C_{GDL} = 6 \text{ mg/mL}$		8	822.4 Pa		0.32 %				
$C_{chitosan} = 0.75, C_{GDL} = 8 \text{ mg/mL}$		2	.598.8 Pa		1.03 %	642 %			
$C_{chitosan} = 1.12, C_{GDL} = 6 \text{ mg/mL}$		3	3850.1 Pa		0.43 %	861 %			

Table 4.3. Parameters associated with BST scaling model, strain breaking, and yield points of matured gels

For alginate-matured gels, the elastic modulus, $G_0 = \lim_{\gamma \to 0} G'(\gamma)$, increases with Ca²⁺ concentration. With $[Ca^{2+}] = 3$ mM, the elastic modulus is 12.8 Pa, which increases by one order of magnitude with 7 mM of Ca²⁺. Also, γ_{break} decreases with increasing Ca²⁺; the network formed with $[Ca^{2+}] = 7$ mM requires a lower strain to break the structure than gel formed with 3 mM of Ca²⁺. The physical mechanism to form alginate gels involves the formation of egg-box structures, whose rupture mechanism unzips the two alginate chains [143,146,150]. The fractal dimension of alginate matured gels varies from 1.33 to 1.25 and decreases according to increased quantity of Ca²⁺. These results for higher Ca²⁺ concentration agree well with d_f reported by Posbeyikian *et al.* [152] for alginate beads; they reported a fractal dimension of 1.25 for alginate beads formed in a

divalent cross-linking solution of $[CaCl_2] = 100 \text{ mM}$ using a SAXS experiment. The dependence of the fractal dimension with Ca^{2+} concentration suggests that the network self-similarity changes slightly with the quantity of Ca^{2+} added. It can be interpreted that the fractal dimension is how the space is filled; for larger d_f , the space is more crowded. Larobina et al. [143] reported that alginate gels formed with $CaCO_3$ by slowly hydrolyzing using GDL, forming multi-unit egg-box structures, resulting in rod-like junctions and bundles of several chains. So, for a fixed concentration of alginate chains, the space is being filled according to the increased quantity of Ca^{2+} with multi eggbox structures. Figure 4.30 shows a cartoon of network structures with different [Ca^{2+}].



Figure 4.30. Cartoon about egg-box structure between alginate chains and the influence of $[Ca^{2+}]$ in their structure.

For chitosan matured gels, the elastic modulus increases with the drying temperature; at 20 °C, G_0 = 16.6 Pa, while at 25 °C, G_0 increases to 43.5 Pa. Also, γ_{break} decreases with temperature increases. Here, controlling the drying speed makes it possible to obtain gels with different rheological behavior; for higher temperatures, the elasticity of chitosan gels increases. The decrease of γ_{break} with the temperature suggests that this higher resistance to strain at lower temperatures is due to more significantly larger hydrophobic cross-linking regions contrary to higher temperatures. Fractal dimension decreases with drying temperature; $d_f = 1.49$ and 1.33 for 20 °C and 25 °C, respectively. Following the concept of fractal dimension and its relationship with space-filling, the matured gel formed at 20°C using chitosan has the most extensive network. This aligns with the notion that the hydrophobic regions are larger at this temperature. However, the corroboration of these hypotheses needs one of the most exhaustive studies to find the relationship between the dry rate of chitosan solution and its network structure. The literature includes several reports about chitosan gel in an alcoholic medium [147,148]. However, the influence of the drying process is not fully understood fully.

The rheological behavior of Alg/Chit matured gels differs concerning gels made from the polysaccharides separately. At low shear stress, the viscoelasticity moduli are independent of γ (linear response), followed by a decrease as γ increases; here, $G'(\gamma)$ and $G''(\gamma)$ do not show an overshoot after the linear viscoelasticity region. The red arrows into the Fig. 4.29 c-d indicate a first yield point characterized by a maximum shear stress, which marks the viscoelasticity yield point followed by a rapid decrease in $G'(\gamma)$. For some Alg/Chit gels, the nonlinear viscoelasticity region shows a second yield point, as marked by the blue arrow. For Alg/Chit matured gels with charge equivalence $(N_{e^-}/N_{e^+} \approx 1, C_{chitosan} = 1.2 \%)$, the presence of two yielding points are notably (see Fig. 4.29 c-d). Here, the final structure can scatter light, as shown in Fig. 4.29d, indicating the formation of large structures of alginate and chitosan chains through PECs formation. Recent works [74,75] have reported the nonlinear viscoelasticity spectra of dense microgel suspensions, which have two yield points as a rheological signature. Saisavadas et al. [74] reported two yield points for thermo-responsive poly(N-isopropylacrylamide) (PNIPAM) core-shell analogous to soft-spheres colloidal glasses with attractive interactions. They concluded that coreshell PNIPAM microgel yields in two steps: one due to the breaking and reformation of entanglements between dangling polymer chains and the second one due to cage breaking and reformation. Lara-Peña et al. [75] reported a similar phenomenology for core-shell microgel dense suspension (PNIPAM as a core and thin poly(ethylene glycol) as a shell). So, due to the similarity of the nonlinear rheological behavior of Alg/Chit matured gels with dense suspension of microgels, it is suspected that the Alg/Chit mature gel shown here consists of soft spheres interconnected between them by dangling polymer chains.

The work of Varadarajan *et al.* [153] supported the idea of the presence of large structure immersed in Alg/Chit gel. They investigated the changes in the structure of alginate/chitosan PECs by adding NaCl; PEC formation was similar to here, showing the same capacity to scatter light. The SASX spectrum for undoped PEC (1:1 volume ratio for alginate and chitosan at 1 w/v %) shows two fractal dimension masses. At q > q_c (~ 0.0143 A⁻¹), d_f^(mass) = 2.2, which attributed to the structure formation upon complexation of alginate and chitosan, and for q < q_c, d_f^(mass) = 4.4, which indicates

the formation of larger aggregates of such polyelectrolyte complexes. Remembering the gel formation protocol, where the PEC formation starts by slowing the acidification of chitosan dispersed in an alginate solution; here, the small chitosan particle could serve as a nucleation point for microgel formation.

The observed nonlinear rheological behavior for $N_{e}-/N_{e^+} \approx 1.5$ (Fig. 4.29c-d, $C_{chitosan} = 1.2$ %) is not simple phenomenon. It has the same rheological signature as for a ratio $N_{e^-}/N_{e^+} \approx 1$ for $C_{GDL} = 8$ and 18 mg/mL with lower elastic modulus; the structure is similar to ratio $N_{e^-}/N_{e^+} \approx 1$ but weakness and more susceptible to break. For $C_{GDL} = 6$ mg/mL, nonlinear rheological behavior presents one yield point similar to a ratio of $N_{e^-}/N_{e^+} \approx 3$, where, according to Fig. 4.29e show one phase is rich in polymer and another one poor in polymer. For a ratio $N_{e^-}/N_{e^+} \approx 3$, there is an excess of negative charges, so neutral PECs formation is impossible. In this case, the first yield point is $\gamma = 0.78$ %. Here, the network structure is very susceptible to breakage because the individual microgels are not yet completely formed, and the union between soft microspheres is softer, contrary to when the $C_{chitosan}$ increases; here, the strain of the first yield point increases.

Figure 4.31 shows AFM images of the dry surface of Alg/Chit matured gels at room temperature for two values of C_{hitosan} (0.75 v/w % and 1.12 v/w %). For C_{chitosan} = 1.12 v/w %, structures on the two largest scales can be seen. For the scale of *600x600 nm*, it is possible to appreciate several pseudo-spherical structures linked together like a necklace of pearls, supporting the idea that the macroscopic structure of Alg/Chit gel is formed by soft spheres interlocking; the radius of one colloid can be estimated in ~ 50 nm. However, this hypothesis should be corroborated by scattering experimental techniques. For example, the SANS experiment will obtain the structure of Alg/Chit's microspheres, and the reflectometry experiment will obtain a density profile through the shell-to-core microgel structure. Comparing the AFM's image series, it can be seen that for the ratio of $N_e^-/N_e^+ \approx 1$ the structures are more compact than a ratio of $N_e^-/N_e^+ \approx 1.5$, showing that the interaction for a stoichiometrically charged system is stronger concerning a negatively charged excess.



 $C_{alginate} = 0.9w/v \% + C_{chitosan} = 0.75w/v \% \qquad C_{alginate} = 0.9w/v \% + C_{chitosan} = 1.12w/v \%$

Figure 4.31. Photographs of dry surface of Alg/Chit matured gels by AFM technique.

Kinetic of gelation: Mean square displacements of microspheres embedded in polysaccharides solutions. The gelation kinetics process is followed by measuring the MSD of PS microspheres (784 nm) embedded in polysaccharide solutions using the DWS technique according to the gelation protocol (see section 4.2.2.3). The MSDs of the microspheres depend on the viscoelastic environment, which evolves from a sol to a gel state as time elapses. Similarly, to gelatin solution, the elapsed time since starting gelation will be named t_q .

Figure 4.32a shows the MSDs of microspheres embedded in alginate solution at 1.8 w/v % at 25 °C. An experimental MSD curve can be fit with a power law with two terms ($\langle \Delta r^2(t) \rangle =$
$At^{\alpha_1} + At^{\alpha_2}$). At short times ($t \leq 5 \times 10^{-3}s$), the first slope is ~ 0.28, showing a clear deviation from the simple diffusive case ($\langle \Delta r^2(t) \rangle = 6Dt$); here, the interaction of particles with the polymer chains limited their displacements with a predominant elastic component where for long times ($t \geq 5 \times 10^{-3} s$) approaches a simple diffusive case because the particle escapes of the interaction with the polymer chains that limited its displacements. This behavior has been reported in polyethylene oxide [154], and acrylamide solutions [133]. Figure 4.32b shows the viscoelasticity spectra from MSDs according to the experimental procedure described in Chapter 3. Here, microrheology and mechanical rheology show good agreement; also, the rheological behavior at high frequency is similar to that of synthetic polymer systems [133,154].



Figure 4.32. Microrheology of alginate solution with $C_{alginate} = 1.8 \text{ w/v} \%$ at 25 °C. a) MSD vs time for microspheres embedded in alginate solution (dia. 784 nm and vol. fraction 0.025); open circles correspond to experimental MSD data, and continuous lines correspond to best fitting using power law with two terms. b) High-frequency viscoelasticity spectra from MSD and mechanical rheometry measurements. Inset: correlation function of the field.

For alginate solution with $[CaCO_3] = 3 \text{ mM}$ (see Fig. 4.33a) at $t_q \leq 42 \text{ min}$, the particles move in a polymer solution as described in the last paragraph, showing two slopes at short and long times. As the gelation process progresses, the slope decreases for a long time as t_q increases. For $t_q \sim 220 \text{ min}$, the MSD curve begins to form a shoulder; the shoulder is more pronounced for $t_q \geq 270 \text{ min}$. From a mesoscopic point of view, the spreading to long times of the first slope (at short times) suggests that the particle cannot move freely as in a simple diffusion case because the polymer chains trap it. A shoulder in MSD curves is evidence of partial particle confinement; here, the

particles explore all the available volume in the polymer network cage formed around it, limiting the displacement of the microsphere similar to those described in gelatin physical gels, where the particle to explore a larger space as time elapses. For alginate solutions with $[CaCO_3] = 7 \text{ mM}$, MSD curves have a similar behavior concerning lower Ca^{2+} concentration; here, the shoulder at long times appears at $t_q \sim 80 \text{ min}$, suggesting the gel state occurs before that $[CaCO_3] = 7 \text{ mM}$. For $t_q >$ 80 min, the shoulder is more pronounced than the alginate solution with $[CaCO_3] = 3 \text{ mM}$ and appears for shorter times. The last observation suggests that the network is narrower, limiting the movement of microspheres.



Figure 4.33. MSD vs. time for microspheres embedded in alginate solution after starting the gelation process at 25 C: a) With $[CaCO_3] = 3 \text{ mM}$, and b) with $[CaCO_3] = 7 \text{ mM}$.

4.3.4. Conclusions

This last part of PhD project explores in detail the linear and nonlinear viscoelastic behavior of different physical gels made from polysaccharides: alginate, chitosan, and their mixture. The Chambon & Winter criteria show a similar critical exponent near the gel point for all physical gels, $n \sim 0.6$, demonstrating that, regardless of the physical mechanism of gelation, the percolation threshold associated with gel network formation is the same. The same critical exponent for physical gelatin gels supported this observation of the similarity of polymeric critical physical gels.

Using the LAOS protocol, the strain-hardening behavior of the matured gels was a focal point of our investigation. The BST scaling model was employed to interpret the results. We found that $d_f \sim 1.25$ -1.33 for alginate gels, and its value is dependent on Ca²⁺ concentration. As the concentration of Ca²⁺ increases, the fractal dimension decreases, indicating that the increased quantity of Ca²⁺ fills the space with multi-egg-box structures, a novel finding in line with Larobina's work [143]. For chitosan gels, $d_f \sim 1.33 - 1.49$, and their value is influenced by the rate at which the modification of the hydrophobicity of the medium by the drying solvent process occurs; at a high rate, df decreases. However, the influence of the dry process is not fully understood yet, presenting an intriguing area for further research. In the case of the alginate/chitosan mixture, the nonlinear rheological behavior exhibits a single yield point for a system with an excessive negative charge $(N_{e^-}/N_{e^+} \approx 3)$ into the alginate chain. For a neutral system $(N_{e^-}/N_{e^+} \approx 1)$, the nonlinear rheological behavior shows two yield points; this is a rheological signature CS-microgels due to similarity with recent works about synthetic CS-microgels [74,75]. AFM micrographs show a structure pearl-necklace-like with a radius of bead \sim 50 nm. The Lissajous-Bowditch curves differ for all gels in the nonlinear viscoelasticity region (not shown); they serve as a unique fingerprint, further adding to the novelty of our findings.

The DWS microrheology kinetics study indicates that the mean square displacement (MSD) decreased as the network formed, and its shape changed based on the physical gelation mechanism. The MSD data allows observation of the various relaxation modes of the chains within the network.

5. General conclusions and perspectives

In this work, three different systems were studied to find a correlation between linear and nonlinear viscoelasticity behavior with their mesoscopic structure of thread-like systems from an experimental physics point of view. The systems were WLMs solutions as a model of living polymers, gelatin solutions as a model of flexible biopolymer, and alginate and chitosan as a model of charged biopolymer; all systems present a phase transition under different conditions. Living polymers preset a transition from isotropic to nematic state when applying shear strain, called the *shear banding phenomenon*. For biopolymers, the system presents a sol-gel transition by physicochemical environment modification. The following is a brief description of the principal finding of this PhD research:

- ★ Linear and nonlinear viscoelasticity of living polymers. Concerning the living polymer system, different experiments were made: flow curves, rotating the internal or external cylinder in a Taylor-Couette geometry, small and large oscillatory shear tests, and linear shear banding observations, all of them to determine how elastic forces modify the rheological behavior in different WLMs solutions. From MSDs of microspheres embedded in the micellar fluids, it was possible to obtain mesoscopic scales' characteristics, particularly the micelles' total contour (*L*_c) and the entanglement length (*l*_e). The ratio $\kappa = L_c/l_e$, called the entanglement index, is related to the entangled micellar network, which is proportional to the system's elasticity. In this sense, the entanglement index, a structural parameter, is valuable for understanding the origin of the contribution of elastic forces, such as continuum macroscopic parameter (the elasticity as the ratio between *Wi* number and *Re* number), from a molecular point of view in the fluid dynamics WLMs solutions. This was the first time where showed a direct relationship between a structural parameter with a macroscopic fluid parameter.
- Linear and nonlinear viscoelasticity of flexible biopolymer gels. In this part of the project, there are different rheological measurements in physical, chemical, and hybrid gelatin gels: linear viscoelasticity spectra around the gel point and nonlinear viscoelasticity spectra in matured gels. Their gel fractal dimensions (*d_f*) were obtained using the BST-scaling model, where for hybrid gels, *d_f* is between 1.46 and 1.60, depending on the dominant crosslinking process. The main features of the Lissajous–Bowditch curves were determined for maturated gels that

follow different gelation processes. The gelation kinetics process is followed by measuring MSDs of microspheres embedded in gelatin solutions using diffusion wave spectroscopy, which in turn allows evaluation of the viscoelastic moduli; the persistence length and the mesh size as a function of time were estimated throughout the gelation process. As time elapses after gelation starts, the persistence length of the unstructured, non-bonded flexible polymer sections decreases due to the formation of bonds. Also, the time evolution of the gel network's mesh size roughly follows an exponential decay. This work shows a dependence of the fractal dimension with the type of crosslink for biopolymer gels and the impossibility of separating the physical and chemical gelation mechanism when both are running simultaneously.

Linear and nonlinear viscoelasticity of flexible charged biopolymer gels. Similar to the last system, different rheological measurements were made in physical gels made with alginate and chitosan and their mixture: linear and nonlinear viscoelasticity spectra around the gel point and matured gels, respectively. The Chambon & Winter criteria show a similar critical exponent near the gel point for all gels n \sim 0.6, demonstrating that the percolation threshold of the network is the same independent of gelation mechanisms. The same critical exponent for physical gelatin gels supported this observation of the similarity of polymeric critical physical gels. For alginate gels, $d_f \sim 1.25 - 1.33$, and its value depends on [Ca²⁺] showing slightly different forms of complexation between Ca²⁺ and alginate chains. For chitosan gels, $d_f \sim 1.33$ -1.49, the value depends on the rate at which the modification of the hydrophobicity of the medium. Surprisingly, the nonlinear viscoelasticity is drastically different concerning gels formed by alginate or chitosan chains. For the polymer mixture, electrostatic interaction leads to the formation of PECs and, under the physicochemical conditions and protocol preparation presented in this work, forms a microgel confirmed by the nonlinear viscoelasticity spectrum and AFM images. In a kinetic study, the MSDs of the microspheres embedded in the polymer matrix decreased according to the formation of the network, and the shape of the MSD's curve was different according to the gelation mechanism.

For the three thread-like systems, this research has revealed a crucial insight that the structure of the polymer matrix directly influences the nonlinear rheological response. The Lissajous-Botwich curves, in particular, serve as a fingerprint for each system. For living polymers, a significant discovery was the definition of the entanglement index, a mesoscopic parameter essential for understanding the entanglement of the micellar network. This index is directly linked to the solution's elasticity from a dynamic fluid point of view. However, the correlation between this index parameter, elasticity, and the inertia-elastic instabilities is a complex and interesting topic that requires further exploration, where the findings shown here should be considered.

The study of large shear strain rheological behavior in biopolymer gels indirectly reveals the fractal dimension, which agrees with the dimension obtained directly from other experimental techniques such as SANS and SASX. An important finding is that the Chambon & Winter criteria show a similar critical exponent (n \sim 0.6) for all physical gels, indicating that the percolation threshold of the network is independent of gelation mechanisms, this suggests a universality of gel-sol transition. In the literature, several critical exponents have been reported for physical gels where the values have a high dispersion. The critical exponents shown here were determined using the same measurement protocol that supported the conclusion about the universality of the physical gelation phenomena. However, additional theoretical work and simulations are needed to conclude the gel-sol transition for biopolymer gels. This study's chemical and hybrid gels were made using glutaraldehyde, a toxic component. Therefore, the next step in the technological approach to hydrogels is to characterize chemical and hybrid gels made with non-toxic components.

An exciting result shows that the mixture of alginate and chitosan forms microgels, possibly due to the gel preparation method and the physicochemical parameters presented here. This mixture exhibits the same rheological signature as core-shell synthetic microgels. Recently, microgels made with natural polymers have gained attention in the soft matter community due to biotechnological applications like Pickering emulsion; the alginate/chitosan microgels shown here could be used for these propourse. It is essential to understand the mesoscopic structure of these microgels, and characterization techniques such as SANS, SASX, and reflectometry can help clarify their structure. Modifying the polymer properties, *e.g.*, molecular weight, deacetylation of chitosan, and M/G proportion of alginate, could result in different microgels. Also, studying the impact of the physicochemical environment is essential to achieving tunable microgels, *e.g.*, PNIPAM-microgels with temperature sensitivity.

6. Scientific technical reports

6. 1 Published work

- López-Santiago, R. F., Delgado, J., & Castillo, R. (2024). Competition among physical, chemical, and hybrid gelation mechanisms in biopolymers. *Soft Matter*, **20**, 2518 – 2531. DOI: <u>https://doi.org/10.1039/D3SM01682J</u>
- López-Santiago, R. F., Delgado, J., & Castillo, R. (2022). Micellar entanglement and its relation to the elastic behavior of wormlike micelle fluids. *Journal of Colloid and Interface Science*, 626, 1015 – 1027. DOI: <u>https://doi.org/10.1016/j.jcis.2022.07.003</u>
- Tavera-Vázquez, A., Rincón-Londoño, N., <u>López-Santiago, R. F.</u>, & Castillo, R. (2021). Measuring mesoscopic scales in complex fluids embedded with giant cylindrical micelles with diffusing wave spectroscopy micro-rheology. *Journal of Physics: Condensed Matter*, *34*(3), 034003. DOI: <u>https://doi.org/10.1088/1361-648X/ac2c3e</u>

6. 2 Manuscripts in preparation

- López-Santiago, R. F., & Castillo, R. Linear and nonlinear viscoelasticity of gels made with polycations, polyanions, and their mixtures; rheology and microrheology approach (in preparation).
- López-Santiago, R. F., & Castillo, R. From gel birth to gel breakdown in alginate solutions: Linear and nonlinear rheological and microrheological approach. Manuscript under review from authors for a special issue, "Kitchen Flows 2024", Physics of Fluids.

7. References

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