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RHÉOLOGIE DES SOLUTIONS DE CHITOSANE ET DES HYDROGELS DE CHITOSANE-XANTHANE

(Rheology of chitosan solutions and chitosan-xanthan hydrogels)

Thèse de doctorat de génie (Ph. D.)
Spécialité: génie chimique

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RÉSUMÉ

Dans ce travail, on analyse les propriétés rhéologiques des solutions de chitosane et des hydrogels de chitosane/xanthane. Les résultats expérimentaux et la modélisation sont présentés et discutés en termes des propriétés moléculaires.

Le chapitre 1 énonce les objectifs de ce travail scientifique. Les chapitres 2 et 3 présentent l'étude rhéologique des solutions de chitosane. Les paramètres expérimentaux contrôlés sont: la température (25, 30, 37 et 45°C), la concentration du chitosane (0.5, 1.0, 1.5 et 2.0%), le type de milieu solvant utilisé pour la dissolution du chitosane (solutions aqueuses d'acide acétique, chlorhydrine et lactique), ainsi que l'effet de la force ionique induit par l'ajout de sel (solution de NaCl 0.2M).

On a réalisé des mesures rhéologiques à l'aide de deux rhéomètres: un rhéomètre à déformation imposée avec une géométrie de cylindres coaxiaux (Contraves Low-Shear) et un rhéomètre à contrainte imposée avec une géométrie cône-plan (Bohlin CVO). Les expériences effectuées ont permis de déterminer la viscosité et la différence des contraintes normales primaires, ainsi que les modules dynamiques G' et G". Les résultats obtenus sont représentés et discutés à l'aide de courbes maîtresses en utilisant des modèles classiques (le modèle de Cross pour les expériences de cisaillement en régime permanent et le modèle de Maxwell pour les expériences de cisaillement en mode oscillatoire), et certaines règles empiriques (les règles de Cox-Merz et de Laun) pour la viscosité, la différence des contraintes normales primaires et les modules dynamiques.

Pour les conditions expérimentales étudiées, les solutions de chitosane se comportent comme des fluides non-Newtoniens rhéofuidifiants avec la présence de contraintes normales. Les solutions montrent un plateau Newtonien à basse vitesse de cisaillement (ou fréquence) et une région non-Newtonienne à haute vitesse de cisaillement. La valeur des fonctions matérielles diminue avec un accroissement de la température et une diminution de la concentration du chitosane (ces fonctions montrent une tendance vers un comportement Newtonien). Nous avons aussi remarqué un effet pour l'anion pouvant être relié avec des effets stériques et électrostatiques. Le paramètre qui affecte la rhéologie des solutions de
façon plus importante est l’addition d’un sel, en particulier pour les systèmes préparés avec un acide faible.

Les chapitres 4 et 5 présentent les résultats expérimentaux et un modèle rhéologique pour les hydrogels de chitosane/xanthane. Dans ce cas les paramètres expérimentaux contrôlés sont: la concentration de l’hydrogel (5, 6, 7, 8, 9, et 10% en poids), la taille de particule de l’hydrogel sec (125-75 μm et 75-55 μm), le solvant utilisé pour la dispersion de l’hydrogel (eau distillée, solution de NaCl 0.05M et une solution tampon de phosphate à pH = 7.0), le "temps d’attente" (le temps entre la préparation du gel et le début des mesures rhéologiques), et aussi le poids moléculaire et degré de acétylation du chitosane.

Pour l’hydrogel on a réalisé des mesures rhéologiques à l’aide d’un rhéogoniomètre Weissenberg à déformation imposée avec une géométrie de plaques parallèles (Bohlin CVO). On rapporte aussi le module de conservation (G’) en tant que fonction de la fréquence et du temps. On rapporte également le seuil de contrainte avec la réalisation d’un test de cisaillement en régime permanent qui mesure la déformation à différentes vitesses de cisaillement. Les résultats sont présentés et discutés avec l’aide du modèle de Gabriele et al., qui est un modèle simple qui donne une relation entre les caractéristiques microscopiques du milieu continu et les caractéristiques macroscopiques.

À partir des résultats obtenus, il a été remarqué que les hydrogels de chitosane/xanthane se comportent comme des gels faibles où leurs modules sont quasi linéaires dans l’intervalle de fréquence mesuré. On note un accroissement du module de conservation avec un accroissement de la concentration des hydrogels. On note également un effet du solvant utilisé pour la dispersion de l’hydrogel. Cet effet est relationné avec l’équilibre électrostatique, où la présence des contre-ions modifie la structure interne des hydrogels. L’effet est observé comme un accroissement des modules et de la viscosité des hydrogels.
ABSTRACT

This work has focused on the rheological properties of chitosan solutions and chitosan-based hydrogels. Experimental results and mathematical modeling are presented and discussed in terms of molecular properties.

Chapter 1 states the scientific objectives of the work and the reasons underlying these objectives. Chapters 2 and 3 present an exhaustive study of chitosan in solutions. The experimental parameters investigated have been: temperature (25, 30, 37, and 45°C), chitosan concentration (0.5, 1.0, 1.5, 2.0% w/w), acid present in the aqueous chitosan solution (acetic, lactic, and hydrochloric), as well as the effect of ionic strength via salt addition (0.2M NaCl solution).

Rheological measurements in solution have been performed using two rheometers: a controlled-deformation rheometer with a coaxial cylinder geometry (Contraves Low Shear) and a controlled-stress rheometer with cone-and-plate geometry (Bohlin CVO). Viscosity and normal forces have been determined as well as the dynamic moduli $G'$ and $G''$. The results are presented and discussed with the help of master curves using classical models (Cross model for steady-shear tests and Maxwell model for oscillatory-shear tests) and empirical rules (Cox-Merz and Laun rules) for viscosity, normal forces, and oscillatory modules.

For the experimental conditions studied, chitosan solutions behave as non-Newtonian shear-thinning fluids with normal stresses: they show a low-shear rate (or frequency) Newtonian region and a high-shear rate (or frequency) non-Newtonian region. As expected, all material functions decrease with an increase in temperature and a decrease in chitosan concentration (tending to a Newtonian behavior). An effect of the anion is also found and can be related to electrostatic and steric effects. The most important parameter affecting the rheology of the solutions was found to be salt addition, especially in systems for which weak acids were used to dissolve the chitosan.

Chapters 4 and 5 present experimental results and rheological modelling for chitosan/xanthan hydrogels in aqueous media. The experimental parameters investigated have been: hydrogel concentration (5, 6, 7, 8, 9, and 10% w/w), range of particle size of the dry hydrogel (125-75
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Figure 5.1 Storage modulus for chitosan A/xanthan hydrogel with particle size range between 75 and 55 μm, at different dispersion media for 7.0 and 10.0% hydrogel concentration. Waiting time = 90 minutes and T = 25°C. [Chitosan A: M_w = 8.2 x 10^5, DA = 28%].................................................. 166

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Table 5.3  Gabriele et al. model parameters for the chitosan B/xanthan hydrogels................................................................. 167
NOTATION

A  Fouss model parameter (eq. 3.17).
    Arrhenius relationship parameter (eq. 3.20).
    Gel strength (5.10 and 5.11)
AF  Gel strength (chapter 5).
a  Power law parameter (eq. 3.37).
ac  Mark-Houwink exponent from intrinsic viscosity.
aT  Shift factor due to temperature effects.
A, A1, A2  Empirical constants for the polymer concentration effect (eqs. 3.11-3.14).
A1  Weltmann model parameter (eq. 3.31).
A2  Hahn model parameter (eq. 3.32).
B  Relaxation time for the Powell-Eyring model (eq. 3.9).
    Empirical constant for the polymer concentration effect (eqs. 3.11-3.14).
    Fulcher model parameter (eq. 3.21).
    Coefficient of thixotropic breakdown with time (eq. 3.24).
B1  Weltmann model parameter (eq. 3.31).
B2  Hahn model parameter (eq. 3.32)
    Mark-Houwink exponent from sedimentation coefficient.
C  Concentration.
c  Constant (eq. 4.7).
C0  Concentration reference.
    Critical concentration (section 4.3.3.2).
C1, C2  Parameters for polymer concentration effect models (eq. 3.13).
C*  Overlap concentration.
C**  Critical concentration.
D  Diffusion coefficient.
DA  Degree of acetylation.
E  Activation energy.
    Elastic modulus.
    Active energy for flow (eq. 3.58).
F  Force required to keep the tip of cone in contact with circular plate for cone
    and-plate rheometers.
f  Frequency.
G  Dynamic shear modulus.
Ga  Equilibrium shear modulus (eq. 5.9).
g  Energy involved in chain strain (eq. 5.9).
G(t)  Stress relaxation.
G*  Elastic modulus.
G'  Storage modulus.
G"  Loss modulus.
Gk  k-th module parameters for the generalized Maxwell model.
G1, G2, G3  Moduli parameters for the generalized Maxwell model.
GPC  Gel Permeation Chromatography.
h  gap for parallel plates rheometers.
J(t)  Creep compliance.
K  Viscometric parameter in the Mark-Houwink equation (eq. 2.5).
    Consistency factor for the power-law model (eq. 3.1).
    Bingham model parameter (eq. 3.2).
K, K1  Tiu and Boger model parameters (eq. 3.8, 3.26, 3.27).
\( \alpha \)  
Exponential viscometric parameter in the Mark-Houwink equation.
Degree of swelling (eq. 4.13).

\( \beta \)  
Angle between cone and plate for the cone-and-plate rheometers (section 2.5.2.2).
Powell-Eyring model parameter (eq. 3.9).

\( \varepsilon \)  
Mark-Houwink exponent from diffusion coefficient.

\( \dot{\gamma} \)  
Shear rate.

\( \dot{\gamma} \)  
Rate-of-strain tensor.

\( \dot{\gamma}_R \)  
Shear rate at the rim of the plate for parallel plates rheometers.

\( \dot{\gamma}_s \)  
Constant shear rate (eq. 3.33).

\( \eta \)  
Viscosity.

\( \eta_s \)  
Solution viscosity (eq. 3.16).

\( \eta_0 \)  
Solvent viscosity (eq. 3.16).

\( \eta_{t_1}, \eta_{t_2} \)  
Viscosities at times \( t_1 \) and \( t_2 \) (eqs. 3.24 and 3.25).

\( \eta_0 \)  
Zero-shear-rate viscosity.

\( \eta_\infty \)  
Viscosity at high shear rates.

\( \eta^* \)  
Infinite-shear-rate viscosity.

\( \eta' \)  
Complex viscosity.

\( \eta'' \)  
Pure viscosity, real part of the complex viscosity.

\( \eta''' \)  
Elastic viscosity, imaginary part of the complex viscosity.

\( \eta_k \)  
k-th viscosity parameters for the generalized Maxwell model.

\( \eta_{rel} \)  
Relative viscosity.

\( \eta_{sp} \)  
Specific viscosity.

\( [\eta] \)  
Intrinsic viscosity.

\( \lambda \)  
Characteristic time for the Carreau model (eq. 3.10).
Structural parameter for Tiu and Boger model (eqs. 3.26 and 3.27).
Relaxation time (eq. 4.14).

\( \lambda_0 \)  
Time constant for the Zimmak model (eqs. 3.29 and 3.30).

\( \lambda_\infty \)  
Equilibrium structural parameter for the Tiu and Boger model (eqs. 3.8, 3.26, and 3.27).

\( \lambda_k \)  
k-th relaxation times for the generalized Maxwell model.

\( \lambda_1, \lambda_2, \lambda_3 \)  
Relaxation times for the generalized Maxwell model.

\( \lambda_1 \)  
Relaxation time for the Maxwell model

\( \lambda_1, \lambda_2 \)  
Oldroyd-B model parameters (eq. 3.33).

\( \mu \)  
Ionic strength.

\( v \)  
Mark-Houwink exponent from radius of gyration.

\( \pi_\tau \)  
Normal pressure on cylinder for Couette rheometers.

\( \pi_{90} \)  
Pressure in the plate for cone-and-plate rheometers.

\( \rho \)  
Density.

\( \sigma_0 \)  
Initial stress (section 4.4.4.2).

\( \sigma_f \)  
Final stress (section 4.4.4.2).

\( \tau \)  
Shear stress.

\( \tau \)  
Stress tensor.

\( \tau_e \)  
Equilibrium shear stress.

\( \tau_0 \)  
Yield strain.

\( \omega \)  
Angular frequency.

\( \omega_c \)  
Crossover frequency (eq. 4.14).
Together with this thesis there is also an appended CD disk. This disk is named: AgustinMartinez_RheoChitoxan_1997-2001.

In the appended CD we present:

- The electronic version of this work. This version can be read and visioned with the Microsoft Word software.
- The results of the entire experimental tests made with chitosan solutions and chitosan/xanthan hydrogels (data and graphics). These results can be read and visioned with the Microsoft Excel software.

The files of the electronic version of the thesis are numbered in agreement with the chapters numbers used in this work.

There are two indexes: for the chitosan solutions results (Chitosan Solutions - Index.xls), and for the chitosan/xanthan hydrogels results (Chitosan/Xanthan Hydrogels - Index.xls). In each index it is possible to find the files and spreadsheets corresponding to the results desired.
1. INTRODUCTION

Chitosan either in solution or in association (as hydrogel) with polyanionic polymers is a versatile biopolymer possessing features such as biodegradability, biocompatibility, bioadhesivity, mechanical resistance, and gelling capacity that makes it attractive in a variety of fields.

A detailed analysis of the relevant rheological properties of chitosan in solution or in chitosan-based gel is important for the establishment of relationships that facilitate and guide their processability and applications.

In the particular case of chitosan solutions and chitosan-based gels for the controlled release of drugs, the study of the relationships between the rheological properties and specific structural and operational parameters is particularly intricate. The reasons are the characteristics of the materials involved and the nature of the interactions that may occur with water and electrolytes as well as the influence of the experimental operational parameters.

In this work a detailed investigation of the flow properties of chitosan solutions and chitosan-based gels at different temperatures, concentrations, solvent media conditions, and chitosan characteristics has been undertaken. The goal was to provide information on the behavior of chitosan either in solutions or associated with xanthan as a hydrogel, for pharmaceutical applications. In particular, the practical goal was to determine from experiments and models how the hydrogels behave rheologically in flow systems of biological significance (i. e. how the hydrogels “move” in the intestinal fluid).

Data correlation and modeling are essential for interpreting any experimental results. For the chitosan systems it was felt desirable to formulate appropriate analytical expressions to describe the rheological parameters in order to transfer such rheological information to the user as well as to determine the homogeneity in the behavior in gels.

Based on the previous considerations, the objectives of this work were fixed as follows:
a) Study the rheological properties of chitosan solutions and chitosan/xanthan hydrogels, combining experimental parameters that allow a complete description of their rheological behavior.

b) Propose and develop rheological models as well as empirical relationships to explain the microstructural characteristic of the chitosan solutions and chitosan/xanthan hydrogels as a function of its macroscopic properties.

c) Obtain master curves that permit to describe the rheological data on solutions using only a few graphs.

d) Explain how the results obtained for chitosan/xanthan hydrogels can be used in applications of the gels as drug-delivery vehicles in biological systems.

The scope of this thesis can be summarized as follows:

- Chapter 1 presents the introduction to the thesis, explaining the objectives and describing the logic of the different chapters.

- Chapter 2 presents the experimental results for the chitosan solutions. The rheology of these systems is evaluated as function of temperature, chitosan concentration, ionic strength, and acidic solution used for chitosan dissolution.

- Chapter 3 presents the rheological modeling of the chitosan solutions, using models and relationships for each situation studied.

- Chapter 4 presents experimental results obtained with chitosan/xanthan hydrogels as function of hydrogel concentration, molecular weight of the chitosan, hydrogel particle size, waiting time, and dispersion medium.

- Chapter 5 presents the results of the rheological modeling of the chitosan/xanthan hydrogels using rheological models and empirical relationships.

- Chapter 6 presents general and specific conclusions as well as recommendations for future works in this area.
2. FLOW AND VISCOELASTIC PROPERTIES OF CHITOSAN SOLUTIONS

2.1 Summary

Changes in the rheological properties of concentrated chitosan solutions as a function of experimental conditions such as temperature (25 to 45°C), chitosan concentration (0.5 to 2.0%), ionic strength (0 and 0.2M NaCl solutions), and type of anion (acetate, lactate, and chloride ions) were studied. Rheological measurements were conducted with chitosan of 1.08 x 10^6 g/mol in molecular weight of and a 25% degree of deacetylation. The rheological tests include steady-shear tests, oscillatory-shear tests and determination of the normal force. Chitosan solutions behave like a typical non-Newtonian material, showing a low-shear rate (or frequency) Newtonian region and a high-shear rate (or frequency) non-Newtonian region; with a decrease in numerical value of the rheological parameters when is increased the temperature and decreased the chitosan concentration (tending to Newtonian behavior). An effect of the anion is also found and can be related to electrostatic and steric effects. The most important parameter was found to be the salt addition, especially in systems for which weak acid was used to dissolve the chitosan.

2.2 Chitin and Chitosan

2.2.1 Chitin

Chitin is the second most abundant polysaccharide in the world after cellulose. It is a long, unbranched polysaccharide constituted of poly-β-\((1\rightarrow 4)\)-N-acetyl-D-glucosamine.

The difference between chitin and cellulose is in the functional group attached to the second carbon in the cycle: the cellulose has an hydroxyl group while the chitin has an acetyl-amine group. The N-acetylation of chitin is usually incomplete to various extents and exists in three polymorphic structures with various degrees of crystallinity [CONRAD, 1965].
Figure 2.1 Chemical structure of chitin.

Chitin and its derivatives are the only abundantly occurring polysaccharides having basic functionalities; they are present in a large number of life forms, including insects and crustaceans [MUZZARELLI, 1985b]. However, chitin is rarely found alone in nature and is usually complexed or covalently bound to protein in the shells of crabs and lobster [KENNEDY et al., 1977].

Crustacean shell waste from crab, shrimp, lobster, or crawfish, is the richest source of chitin as well as being the only chitin’s source presently available in quantities sufficient to support a commercial chitin/chitosan industry. Crustacean shell waste mainly consist of proteins (30-40%), calcium carbonate (30-50%), and chitin (20-30%). Preparation methods for chitin can be adapted to sources in order to meet compositional differences [NO et al., 1997].

Chitin is usually treated in three basic steps [CONRAD, 1965]:
1- Deproteinisation: Protein separation, generally with dilute sodium hydroxide solution (1-10%) at near boiling temperatures (65-100°C).
2- Demineralisation: Calcium carbonate (and calcium phosphate) separation with dilute hydrochloric acid at room temperature.
3- Decoloration: Removal of pigments with organic solvents or bleaching agents. For example using ethanol and ether, or sodium hypochlorite solutions.

2.2.2 Chitosan

Chitosan is a linear polyether of high molecular weight. Chitosan has unique properties including bioactivity, biocompatibility, and biodegradability favourable for a broad variety of industrial and biomedical applications. Chitosan is an hydrophilic polyelectrolyte obtained by
deacetylation of chitin. It is a polymer of N-acetyl-D-glucosamine partly deacetylated by treating chitin with a concentrated (~50%) solution of sodium hydroxide.

![Chemical structure of chitosan.](image)

**Figure 2.2 Chemical structure of chitosan.**

### 2.2.2.1 Preparation

We can distinguish chitosan from chitin in two ways:

- Only chitosan is soluble in acetic acid and formic acid.
- Nitrogen Percentage: In chitin the nitrogen percentage is lower than 7%; chitosan has more than 7% in nitrogen.

Conversion of chitin to chitosan is generally achieved by treatment with concentrated sodium or potassium hydroxide solutions (40-50%) usually at 100°C or higher to remove some or all of the acetyl groups from the polymer. N-acetyl groups cannot be removed by acidic reagents without hydrolysis of the polysaccharide, thus alkaline methods must be employed for N-deacetylation. During deacetylation proper agitation is needed to obtain uniformity in reaction. This requires a high degree of fluidity in the reaction mixture, i.e., low ratio of chitin solids to alkali solutions (about 1/10) [TECHNICAL INSIGHTS, 1989].

### 2.2.2.2 Chemical Structure

The true crystal structure and configuration of chitosan has been examined by small-angle light scattering (SALS), x-ray diffraction, and polarised light microscopy. Chitosan is highly crystalline in nature and is more rigid than cellulose. Small-angle x-ray diffraction studies carried out on chitosan powders showed regular packing of the molecules in parallel bundles, analogous to that of stretched polyethylene and polypropylene fibrils. The data indicate that
the interactions of chitosan macromolecules along an axis give rise to a fibrous structure [MUZZARELLI, 1977]. Different film fabrication procedures showed a range of x-ray and infrared patterns indicating the possibility that chitosan can exist in different polymorphic forms [SAMUELS, 1981; QURASHI, 1990].

2.2.2.3 Chemical and Physical Properties

Some specifications of the physical and chemical properties are presented in Table 2.1 [MUZZARELLI, 1985b; MUZZARELLI et al., 1998; QURASHI, 1990]. The properties of chitosan are related to the average molecular weight and molecular-weight distribution of the polymer, to the extent of deacetylation, and chain degradation that occurs during the alkaline hydrolysis of chitin.

One of the most important parameter in the characterisation of the chitosan is the degree of acetylation (DA); this degree is defined as the ratio of the number of formed amine groups (NH₂) to the initial number of acetyl-amine groups (NHCOCH₃) present in chitin.

2.2.2.4 Solution Properties

a) Polyelectrolyte Properties

Polyelectrolytes, like chitosan in solutions, are polymers with potentially ionizable groups. In polar solvents they are dissociated into polyions and counterions. The strong electric interaction between them is the source of the characteristic properties of polyelectrolytes in solution.

The ionic interactions in a polyelectrolyte solution have the following characteristics [KINZLE-STERZER, 1984; MUNK, 1989]:

- Polyelectrolytes are polymers formed by monomer units with an ionizable group where the degree of ionization depends on the dissociation constant and on the hydrogen ion concentration of the polymer solution.
- The electrical forces are inversely proportional to the dielectric constant of the solvent.
- The charge density has a strong influence on the conformation of a polyelectrolyte chain. The intermolecular forces (electrostatic interaction between ions) decrease only with the
square of the interionic distance and have profound effect on the conformation of the polymer coils.

<table>
<thead>
<tr>
<th>Specification</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Moisture</td>
<td>*From 2 to 10% w/w under normal laboratory conditions.</td>
</tr>
<tr>
<td>*Nitrogen</td>
<td>*Usually form 7 to 8.4% w/w.</td>
</tr>
<tr>
<td>*Degree of acetylation</td>
<td>*40% in normal chitosan, 0 - 10% in extensively to fully deacetylated chitosan.</td>
</tr>
<tr>
<td>*Ashes at 900°C</td>
<td>*Lower than 1.0%.</td>
</tr>
<tr>
<td>*Molecular weight</td>
<td>*1x10⁵ to 2x10⁶ g/mol.</td>
</tr>
<tr>
<td>*Titration</td>
<td>*With potassium polyvinylsulfonate and by alkalimetry.</td>
</tr>
<tr>
<td>*Dissociation constant, Kₐ</td>
<td>*Between 6.0 and 7.0; most often 6.3</td>
</tr>
<tr>
<td>*X-ray diffraction data</td>
<td>*Typical peaks at 8°58'- 10°26' and 19°58' - 20°00'.</td>
</tr>
<tr>
<td>*Viscosity of 1% w/w solutions in 1% w/w acetic acid</td>
<td>*From 200 to 3,000 cP.; degraded chitosan have lower viscosity.</td>
</tr>
</tbody>
</table>

b) Solubility:
Chitosan is quite insoluble in sodium hydroxide, sodium carbonate, and other bases. However, chitosan powders can be dry blended with an organic acid to give a chitosan acid blend that is self-dissolving. Chitosan in its free amine form (-NH₂) is not usually soluble in water at pH above 6.5 and requires acids such as acetic acid to prepare aqueous solutions. In this case, the free amino group becomes protonated to form cationic amine groups (-NH₃⁺).
The following equilibrium describes the state of ionisation:

\[
\text{Chit-}NH₂ + H₂O⁺ \leftrightarrow \text{Chit-NH₃}^+ + H₂O
\]  

Chitosan is also soluble in nitric acid at pH < 2 with some degradation. Inorganic acids, except sulphuric and phosphoric, in concentrations around 1% generally dissolve chitosan after prolonged stirring and warming [RIANAUDO et al., 1999b].

2.2.2.5 Reactions
Chitosan, being a high-molecular-weight biopolymer, is a linear polyelectrolyte whose reactive amino groups together with primary and secondary hydroxyl groups are readily
available for chemical reactions to alter its mechanical, physical, and solution properties. The possible reaction sites for chemical modifications of chitosan are illustrated in Figure 2.3 [HON, 1996].

2.2.2.6 Uses and Applications

Due to the fact that chitosan has a high molecular weight, a cationic behaviour, and can form films and gels, the material has been extensively used in the industry. Its most popular uses are as flocculant in the clarification of waste water, as a chelating agent for metals in the detoxification of hazardous waste, for the clarification of beverages, and for agricultural purposes such as a fungicide [ILLUM, 1998]. Other chitosan uses are as cosmetic agent [LANG et al., 1988], as anticholesteremic agent [SEO et al., 1992], as coating agent of textile fibers [STRUSZCZYK, 1997], and as medium for ultrafiltration membrane [SAKURAI, 1997].

Several other applications of chitosan as biomedical agent and as excipient in pharmaceutical products are being investigated:

a) Biomedical Applications

High-quality chitosan, after complete removal of potentially toxic and contaminating materials is biodegradable, bioactive, biocompatible, and safe for use; many tests have revealed their safety [MUZZARELLI, 1985b]. Chitosan has been evaluated in a number of biomedical applications like bacteriostatic agent, hemostatic agent, spermicide, dialysis membrane, contact lens, mucosal hemostatic agent, etc. [HON, 1996; FELT et al., 1998; GUPTA et al., 2000].

![Figure 2.3 Possible reaction sites for chemical modification of chitin and chitosan: primary and secondary hydroxyl groups, amino and acetamino groups.](image-url)
b) Drug Delivery Systems

Chitosan, besides having a cholesterol-lowering effect, has many useful physical properties in the delivery of drugs. One of the first considerations leading to the development of biologically active polymers was the hope of achieving controlled release of active substances either in the intestinal tract, transdermal or in mucoses. Due to its unique polymeric and cationic character, and its gel and film forming properties, chitosan should allow the control of the rate of drug administration and prolong the duration of the therapeutic effect as well as the targeting of the drug to specific sites [HON, 1996; ILLUM, 1998].

Chitosan and its derivatives have been usefully employed for the preparation of drug loaded microparticles. Microparticles is a general term used to indicate both microspheres and microcapsules. The first are defined as microparticles in which the drug is uniformly dispersed in the chitosan network, the latter as particles in which a solid core constituted by the drug is surrounded by a polymeric membrane [GENTA et al., 1997; GUPTA et al., 2000].

2.3 Conformation in Chitosan Solutions

2.3.1 Conformation in Polymer Solutions

Conformation refers to the different arrangements of atoms and substituents of the polymer chain in space, brought about by rotations about single bonds. The possible conformations of a polymer chain are function of the chain flexibility, solvent properties, concentration, etc. Conformational studies are important in controlling the rheological properties of polymer solutions and the characteristics of the material prepared.

Parameters frequently used to characterize the conformation of polymer solutions include Mark-Houwink type exponent such as: a, b, ε, and ν. These parameters correlate the intrinsic viscosity ([η]), sedimentation coefficient (s), diffusion coefficient (D), and radius of gyration (Rg), respectively vs. molecular weight of the polymer (M). In general case of polymer solutions the numerical values of these exponents as function of the conformation of polymer are listed in table 2.2 [TSAIH et al., 1997].
The relationships between polymer conformations and the exponents of the Mark-Houwink equation are:

**Table 2.2 Relationship between the Mark-Houwink exponents and the polymer conformation.**

<table>
<thead>
<tr>
<th>Mark-Houwink type equations</th>
<th>Conformation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sphere</td>
</tr>
<tr>
<td>$[\eta] = k \cdot M^a$</td>
<td>0</td>
</tr>
<tr>
<td>$s = k' \cdot M^b$</td>
<td>0.7</td>
</tr>
<tr>
<td>$D = k'' \cdot M^c$</td>
<td>0.3</td>
</tr>
<tr>
<td>$R_g = k''' \cdot M^d$</td>
<td>0.3</td>
</tr>
</tbody>
</table>

**INTRINSIC VISCOSITY:**
Measurements of the intrinsic viscosity are carried out in dilute solutions using a flowing solution in a capillary tube. If $\eta$ is the viscosity of the polymer solutions, expressed in Pa · s, and $\eta_0$ is the solvent viscosity, then:

$$\eta_{rel} = \frac{\eta}{\eta_0}$$  \hspace{1cm} (2.2)

Where $\eta_{rel}$ is the relative viscosity. The specific viscosity is defined from the relative viscosity as:

$$\eta_{sp} = \eta_{rel} - 1$$  \hspace{1cm} (2.3)

The specific viscosity, divided by the concentration yields the intrinsic viscosity when extrapolated to zero concentration:

$$\left[ \frac{\eta_{sp}}{c} \right]_{c \to 0} = [\eta]$$  \hspace{1cm} (2.4)

**SEDIMENTATION COEFFICIENT:**
The sedimentation coefficient ($s$) can be measured using an ultracentrifugation technique coupled with a scanning optical system that shows the position of the sedimentation
In the literature, several studies have been performed on chitosan in solutions:

Roberts and Domszy [1982] reported on the viscometric constants $a$ and $k$ in the Mark–Houwink equation. The parameters were determined for:

- Chitosan in 0.1M acetic acid + 0.2M sodium chloride: $a = 0.93$, $k = 1.81 \times 10^{-3}$.
- Chitosan in 0.1M acetic acid + 0.02M sodium chloride: $a = 1.26$, $k = 3.04 \times 10^{-5}$.

This means that for chitosan solutions in these conditions the polymer conformation is changing from a random coil to a rod conformation.

Pogodina et al. [1986] reports data for:

- Chitosan in (2%) 0.33M acetic acid + 0.3M NaCl: $a = 1.02$, $k = 3.41 \times 10^{-3}$.

In their case the model of the random coil may adequately describe the chitosan solutions with high ionic strength, such as 0.3M NaCl; but this value give some indication that there is a possible transition to a rod-like conformation.

Yomota et al. [1993] determined the viscometric constants for the system:

- Chitosan in 0.5M acetic acid + 0.5M sodium acetate: $a = 0.59$, $k = 1.99 \times 10^{-1}$.

These values correspond to random coil conformation in accordance with the data reported by Tsaih and Chen [1997]: $a = 0.5$ to 0.8 for polymer solution corresponds to a random coil conformation.

Wang et al. [1991] have determined the parameters for chitosans with different degrees of acetylation (DA).

- Chitosan in 0.2M acetic acid + 0.1M sodium acetate: $DA = 31 \%$, $a = 1.12$, $k = 0.104 \times 10^{-3}$.
- Chitosan in 0.2M acetic acid + 0.1M sodium acetate: $DA = 16 \%$, $a = 0.96$, $k = 1.424 \times 10^{-3}$.
- Chitosan in 0.2M acetic acid + 0.1M sodium acetate: $DA = 9 \%$, $a = 0.88$, $k = 6.589 \times 10^{-3}$.
- Chitosan in 0.2M acetic acid + 0.1M sodium acetate: $DA = 0 \%$, $a = 0.81$, $k = 16.80 \times 10^{-3}$.
It is clear that the \( a \) values decrease from 1.12 to 0.81 when the DA decreases from 31 to 0\%. This indicates that the rigidity of chitosan chains in solutions decreases when decreases the DA of chitosan due to the presence of hydrogen bonds between segments in the structure. This means that the system goes from rod-like configuration to a random-coil configuration (less extended configuration), which is consistent with a decreasing value of DA.

Tsaih and Chen [1997] use different chitosan solutions with a constant degree of acetylation (DA = 17\%), but different molecular weight and at 30\°C.

- Chitosan in 0.01M HCl + 0.01M NaCl \( a = 0.715, k = 5.48 \times 10^{-3} \).
- Chitosan in 0.05M HCl + 0.05M NaCl \( a = 0.614, k = 1.05 \times 10^{-3} \).
- Chitosan in 0.10M HCl + 0.10M NaCl \( a = 0.595, k = 1.18 \times 10^{-3} \).
- Chitosan in 0.20M HCl + 0.20M NaCl \( a = 0.569, k = 1.28 \times 10^{-3} \).
- Chitosan in 0.30M HCl + 0.30M NaCl \( a = 0.521, k = 2.04 \times 10^{-3} \).
- Chitosan in infinite ionic strength \( a = 0.335, k = 1.24 \times 10^{-2} \). (Obtained by extrapolation)

The intrinsic viscosity of chitosan decreases with increasing solution ionic strength. The data shows that the \( a \) value decreases with increasing ionic strength. Data also indicate that the conformations of the chitosans in these solutions were random coils and that no conformational transition occurred.

The value at infinite strength indicates that the conformation of chitosan in an infinite ionic strength solution is a compacted sphere.

The chitosan conformation also has been explained with the wormlike model [MUNK, 1989]. In this model the direction of the chains is changing gradually, i.e. the chain has a constant curvature in a tangent plane. However, the orientation of the tangent plane is changing randomly, and the chains resemble a worm. The average projection of the end-to-end vector of the model onto the initial tangent of the curved chain approaches a finite limit as the contour length approaches infinity. This limit is called the persistence length; it is the characteristic parameter of the wormlike model. Rinaudo and Domard [1989] using intrinsic viscosity data for different chitosan solutions found values for the persistence length and their results are in agreement with a wormlike chain model to describe the chitosan solutions.
From these studies we can thus conclude that:

- When the electrostatic repulsion forces are decreased the conformation of chitosan becomes more entangled (from a rod conformation to random coil conformation or a compact sphere).

- The rigidity of chitosan chains in solution decreases with decreasing the DA value of chitosan indicating that the chitosan chain becomes less extended (more entangled like a random coil). Following a deacetylation, the acetyl groups transform partly or completely into amine groups. The nitrogen group is unable to form hydrogen bond due to the protonation of amine group with H⁺, as to reduce molecular rotation. Thus the rigidity decreases and the softness increases to a decreasing DA value.

- In general chitosan in solution adopts a conformation between a rod-like molecule with flexibility and a coiled molecule with a low flexibility. A decrease in the degree of acetylation and an increase in the ionic strength will produce a conformational transition from a rod conformation to a random coil conformation.

2.4 Rheology of Chitosan Solutions: Literature Review

The rheology of chitosan has not been investigated as widely as that of other polysaccharides like xanthan: [WHITCOMB et al., 1978; JAMIESON et al., 1982; ROCHERFORT et al., 1987; CARRIERE et al., 1993; ZIRNASAK et al., 1999]. However there are specific works in chitosan rheology. These works can be classified by the physical form of chitosan used:

a) Rheology of dilute and concentrated chitosan solutions
b) Rheology of films/membranes of chitosan
c) Rheology of gels of chitosan
d) Rheology of mixtures that include chitosan

One reason why chitosan is used in a large variety of engineering and biomedical applications is related to its many useful physical forms. Chitosan powders are usually dissolved and used commercially as aqueous solutions. The later form crystal clear solutions that can be used as the starting point for making films, pastes, sponges, or gels.

Chitosan degrades before melting, which is typical for polysaccharides with extensive hydrogen bonding. This makes it necessary to dissolve chitosan in an appropriate solvent.
The rheological behavior of chitosan, a typical random coil polyelectrolyte, is an important subject of study because most of the uses of chitosan are made possible by the preparation of chitosan solutions. Their overall molecular conformation and the degree of hydrogen bonding between neighbouring chain segments dictate the flow behavior. The intrinsic viscosity [$\eta$] determined for chitosan in solution indicates that chitosan adopts conformations which range from a random coil to a more compact quasi-globular shape [MUZZARELLI, 1985a].

It is known that the intrinsic viscosity of a linear polymer is related to the molecular weight by the Mark-Houwink equation [BIRD et al., 1987]:

$$[\eta] = K M_w^\alpha$$

(2.5)

The K and $\alpha$ parameters depend on the particular polymer-solvent pair and on the temperature. $M_w$ is the molecular weight of the polymer. For random coil polymers the exponent $\alpha$ lies between 0.5 and 0.8, and the overall shape of the hydrodynamic volume is spherical. Exponents larger than 0.8 reflect a highly expanded conformation, which may be rodlike in shape. The parameter K is dependent predominantly on the geometry of the interresidue linkages within the polymer chain [LAPASIN et al., 1995].

Several authors have reported K and $\alpha$ values for chitosan of varying degrees of acetylation (DA), in various solvent systems. J. Z. Knaul [KNAUL et al., 1998] and M. R. Kasaa [KASAAI et al., 2000a] have summarized data for molecular weight and related the constants K and $\alpha$, for different solvent systems and molecular weight ranges of chitosan. The most commonly
used solvent systems are buffer solutions of acetic acid/sodium acetate at various concentrations.

Maghami and Roberts [1988] has shown that for a series of chitosans having the same relative molecular weight and distribution but differing in DA, the same viscometric constants in the Mark-Houwink equation are applicable to chitosan over a DA range between 0 and 40%. They report that the most accurate values of these constants are \( K = 1.81 \times 10^{-3} \text{ cm}^3 \text{ g}^{-1} \) and \( \alpha = 0.93 \) at 25°C using 0.1M CH₃COOH/0.1M NaCl as solvent.

However, Wang [1991] determined the Mark-Houwink parameters for chitosan with different acetylation degrees in 0.2M CH₃COOH/0.1M CH₃COONa at 30°C. The results can be expressed as:

\[
\begin{align*}
\text{DA} = 31\% & \quad [\eta] = 0.104 \times 10^{-3} \: M_w^{1.12} \quad (\text{cm}^3/\text{g}) \\
\text{DA} = 16\% & \quad [\eta] = 1.424 \times 10^{-3} \: M_w^{0.96} \quad (\text{cm}^3/\text{g}) \\
\text{DA} = 9\% & \quad [\eta] = 6.589 \times 10^{-3} \: M_w^{0.88} \quad (\text{cm}^3/\text{g}) \\
\text{DA} = 0\% & \quad [\eta] = 16.80 \times 10^{-3} \: M_w^{0.81} \quad (\text{cm}^3/\text{g})
\end{align*}
\]

(2.6) (2.7) (2.8) (2.9)

It was shown that the values of \( \alpha \) decreased and the values of \( K \) increased when DA values decreased from 31 to 0%. This was explained by a reduction of rigidity of the molecular chain and an increase of the electrostatic repulsion force of the ionic groups along the polyelectrolyte chain in chitosan solutions when the DA of chitosan decreases gradually.

Other proof that the parameters of the Mark-Houwink equation are very sensible to the solvent are the results shown by Kasaa et al. [2000]. They used a 0.25M CH₃COOH/0.25M CH₃COONa solvent where \( K = 1.49 \times 10^{-4} \) and \( \alpha = 0.79 \). This value of \( \alpha \) suggests that the conformation of chitosan in this solvent is that of a flexible chain. The Mark-Houwink equation constants for chitosan with varying DA and solvents of different pH and ionic strength have been summarised by Kasaa et al. (2000). They show that \( \alpha \) and \( K \) are inversely related and depend on the function \([\text{DA}/(\text{pH } \mu)]\); where \( \mu \) is the ionic strength in mol/l.

When different acid solvents were used under different pH values for a given chitosan concentration, the reduced viscosity (specific viscosity/concentration) decreases with an
In general, in absence of structure-induced formation, the rheological behavior of concentrated chitosan solutions is monotonically shear-thinning; the viscosity varies between two extremes the zero-shear-rate viscosity (\(\eta_0\)) and the infinite-shear-rate viscosity (\(\eta_\infty\)); and substantially increases with polymer concentration.

The behavior of low molecular weight (1.3 x 10^6 g/mol) chitosan in concentrated solutions and constant temperature (25°C) has been studied [KIEZLE-STERZER, 1984]. For chitosan solutions (dissolved in HCl) at concentrations between 0.5 and 1.5% w/w the viscosity increases as chitosan concentration increases. At low chitosan concentrations, the viscosity of chitosan solutions is not shear rate dependent; at increasing chitosan concentrations, the viscosity-shear rate relationship shows two regions: one Newtonian region at low shear rate, and another region where increasing the shear rate leads to a viscosity decrease. Intermolecular interactions were found responsible of the increase of the zero-shear-rate viscosity (\(\eta_0\)), at given chitosan concentrations and ionic strength, with increasing pH values.

Mucha [1997] presented results of the rheological characterisation of chitosan solutions of low molecular weight in a wide chitosan concentration range (0.5 to 5.0%). Chitosan powder samples of different acetylation degrees were dissolved in acetic acid solutions and the tests were performed in the temperature range 20 to 41°C. The behavior of the solutions was found to be typical of non-Newtonian shear-thinning fluids. Only in the case of low concentrations they are close to Newtonian fluid behavior.

The same work also showed that the slope of the relationship viscosity-shear rate (power-law parameter) decreases with increasing molecular weight and chitosan concentration, while increases with increasing temperature. The most important effect is the concentration effect; the shear viscosity increases much faster than the concentration of the chitosan solutions. This is caused by an increase of interactions of the chitosan macromolecules leading to their entanglement. The relationship between the power-law parameter versus temperature can be explained by the increase of the chitosan solubility at high temperatures, moving in the direction of more Newtonian flow behavior.

The supermolecular organisation of concentrated solutions of chitosan has been rheologically studied [NUD'GA et al., 1993]. For a 3% solution of chitosan in 2% acetic acid (\(M_w = 1.88 \times 10^5\) g/mol, DA = 22%, and pH controlled using NaOH), it was observed that the structure of
the solutions was determined to a significant degree by the pH of the solution. There is an critical point at pH = 5.2 where the viscosity is the lowest. This behavior is explained by the occurrence of two processes: contraction of the macromolecular coils due to the electrostatic interaction, and formation of associations as a result of hydrogen bonding by the non-protonated amino groups.

The effect of the degree of acetylation has been studied also by Wang and Xu [1994b]. They used 2% w/w solutions of chitosan with DA of 9% and 25% in 0.2M acetic acid at 25 °C. They found that the viscosity and flow properties of the concentrated solutions of chitosan varies with different degrees of acetylation. The viscosities and the non-Newtonian flow properties of the solutions increase with a decreasing degree of acetylation of chitosan.

There are only few works in oscillatory shear rheology for chitosan solutions. Kjoniksen et al. [1997] worked with 1% solutions of chitosan (M_w = 4 x 10^5) in 1% acetic acid in the presence of a surfactant and at different pH values and 25°C. They found that under these conditions the oscillatory properties were essentially not influenced by pH, surfactant, and oscillation frequency.

In this work we will present an experimental study on the rheological behavior of chitosan solutions using a chitosan of high molecular weight (1.08 x 10^6 g/mol). In contrast with other works, the results are obtained with chitosan dissolved in three different acidic solutions, four chitosan concentrations, and four different temperatures to determine their effects on the modification of the counterion concentration. Likewise, the properties are evaluated in steady and dynamic regimes. Besides, the evaluation of the normal stresses for these systems is also performed.

2.5 Experimentation

2.5.1 Materials

The chitosan was made from chitin obtained from shrimp’s shells. It was produced by Kemestrie Inc., Sherbrooke (Quebec).
Analytical grade acetic acid (BDH), lactic acid (Sigma), and hydrochloric acid (BDH) were used for chitosan solubilization. Analytical grade sodium chloride (Sigma) was used to adjust the ionic strengths. For the preparation of all the aqueous solutions, we used distilled water.

2.5.2 Rheometry

We measured the rheology of our samples using two geometries: concentric cylinders (Couette), and cone-and-plate. These geometries produce viscometric flows which are easy to analyse.

2.5.2.1 Coaxial – Cylinder Rheometer (Couette Rheometer)

The Couette geometry allows a straightforward determination of viscosity when the gap between the cylinder is small. For large gaps the analysis is complicated because the velocity field depends on the viscosity function. The hole pressure can cause significant errors in this instrument, making measurement of the normal stress difficult.

The outside cylindrical frame is the reservoir for the fluid. Sufficient fluid is initially poured into the reservoir so that, when the inside assembly is put in place, the fluid will fill completely the gap between both cylinders, with some of the fluid covering the top of the inner cylinder. Corrections for end effects may be applied (see figure 2.5).

Using the equations of motion and force balance, viscosity and normal forces can be obtained using the following relations [BIRD et al., 1987]:

\[ \eta(\dot{\gamma}) = \frac{T(R_0 - R_1)}{2\pi R_1^3 L |\Omega_0 - \Omega_1|} \]  
(2.10)

\[ \Psi_1(\dot{\gamma}) = \frac{-[\pi_n(R_0) - \pi_n(R_1)] R_1}{(R_0 - R_1) \dot{\gamma}^2} + \frac{\rho R_1^2}{3 \dot{\gamma}^2} \left( \Omega_1^2 + \Omega_0^2 + \Omega_1 \Omega_0 \right) \]  
(2.11)

\[ \dot{\gamma} = \frac{|\Omega_0 - \Omega_1| R_1}{R_0 - R_1} \]  
(2.12)
plate can be rotated. For a very small angle, the velocity profile is linear with respect to the angle position in the gap (see figure 2.6).

![Figure 2.6 Cone-and-plate rheometer.](image)

Using a similar approach of the Couette geometry, viscosity and normal forces can be obtained as [BIRD et al., 1987]:

\[
\eta(\dot{\gamma}) = \frac{3T}{2\pi R_0^3 \dot{\gamma}}
\]

(2.13)

\[
\Psi_1(\dot{\gamma}) = \frac{2F}{\pi R_0^2 \dot{\gamma}^2}
\]

(2.14)

\[
\Psi_1(\dot{\gamma}) + 2\Psi_2(\dot{\gamma}) = -\frac{1}{\dot{\gamma}^2} \frac{\partial \pi_{\theta \theta}}{\partial \ln r}
\]

(2.15)

\[
\Psi_2(\dot{\gamma}) = \frac{p_a - \pi_{\theta \theta}(r)}{\dot{\gamma}^2}
\]

(2.16)

\[
\dot{\gamma} = \frac{\Omega \beta}{\sqrt{}}
\]

(2.17)

Where:

- \(\eta\) Viscosity [Pa.s].
- \(\dot{\gamma}\) Shear rate [1/s].
- \(\Psi_1\) First normal stress coefficient [Pa.s^2].
- \(\Psi_2\) Second normal stress coefficient [Pa.s^2].
\( R_0 \)  
Radius of circular plate [m].

\( \beta \)  
Angle between cone and plate [\( ^\circ \)].

\( \Omega \)  
Angular velocity of cone [rad/s].

\( T \)  
Torque on plate [N.m].

\( F \)  
Force required to keep the tip of cone in contact with circular plate [N].

\( \pi_{\theta\theta}(r) \)  
Pressure measured by flush-mounted pressure transducers in the plate [Pa].

\( p_a \)  
Atmospheric pressure [Pa].

2.5.3 Methods

2.5.3.1 Molecular Weight Determination

Two average molecular weights are commonly used, depending on how the contributions of sample chains having the same length are taken into account statistically. If it is with respect to their number fraction in the sample, the number average molecular weight \( M_n \) is obtained; if their weight fractions is considered, the weight average \( M_w \) is obtained. The relationship \( M_w / M_n \geq 1 \) (called polydispersity) holds for all polymers, with the equality representing a monodispersed sample, where all chains have the same molecular weight.

The weight-average molecular weight of chitosan was determined by means of the Gel Permeation Chromatography (GPC) method, also known as Size Exclusion Chromatography (SEC) method. The technique involves dissolving chitosan in an aqueous salt solution (0.2M sodium acetate-0.5M acetic acid). The molecules are separated according to their size using a POPYSEP-GFP-P Linear Column (300 x 7.8 mm) by Phenomenex, California. The detection was made using a refractive index detector.

A sample of the dilute polymer is introduced into a solvent stream flowing through the columns. The polymer molecules can diffuse into the internal structure of the gel to an extent which depends on their size. Large molecules can only penetrate a small fraction of the internal portion of the gel, or are completely excluded. An elution curve is obtained by plotting the amount of polymeric solute against the retention time of the different molecule size. Weight-average degrees of polymerisation are calculated from their elution patterns based on a calibration curve obtained using Pullulans standards [TERBOJEVICH et al., 1997]
The weight-average molecular weight ($M_w$) was determined to be $1.08 \times 10^6$ g/mol and a polydispersity index $M_w/M_n = 3.0$ by GPC method.

2.5.3.2 Degree of Acetylation Determination

The degree of acetylation (DA) was determined by the Infrared Spectroscopy technique. The method involves measuring the absorbance of either the amide I or amide II adsorption band, using the absorbance of a suitable internal reference peak to correct for the film thickness of sample. The method uses the ratio $A_{1655}/A_{3450}$ [MUZZARELLI et al., 1997; ROBERTS, 1997].

$$DA = \left( \frac{A_{1655}}{A_{3450}} \right) \left( \frac{100}{1.33} \right)$$  \hspace{1cm} (2.18)

- $DA$: Degree of acetylation
- $A_{1655}$: Absorbance for NH peak
- $A_{3450}$: Absorbance for OH peak

A spectrophotometer Nicolet FTIR 5 DXB was used. The prototype chitosan used has a degree of acetylation of 25%.

2.5.3.3 Preparation of Chitosan Solutions

a) Chitosan-acid solutions

Chitosan samples were used to prepare solutions of different concentrations (2.0, 1.5, 1.0, and 0.5 wt %); the chitosan was dissolved in aqueous acid solutions containing 1.0% w/w of either acetic acid, lactic acid, or hydrochloric acid.

To obtain a good solution, chitosan powder was dispersed by agitation with a magnetic stirrer until total solubiilisation. The chitosan solutions were kept at 4°C until rheological tests were performed.
b) Chitosan Solution + Salt (NaCl)
To obtain a chitosan solution with an ionic strength of 0.2M in sodium chloride (NaCl), the NaCl solution was added to the acidic solution before the introduction of the chitosan.

2.5.3.4 Rheological Tests

The rheological tests were performed using two rheometers: A Contraves Low Shear LS-30 rheometer for the measure of the steady-shear viscosity at very low shear rates; and the Bohlin CVO HR 120 rheometer for the measure of steady-shear viscosity, the normal force, and the oscillatory-shear viscosity.

a) The Contraves Low Shear LS-30 rheometer is a shear-imposed rheometer. It measures torque to very low rate of steady shear.

For the determination of the viscosity of a sample of the chitosan solution we used a concentric cylinder geometry. The solution was placed into the major cylinder of the LS-30 rheometer. The technical specifications for the selected geometry were:

<table>
<thead>
<tr>
<th>Cylinders Length</th>
<th>21.5 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner Radius ( (R_0) )</td>
<td>3 mm</td>
</tr>
<tr>
<td>Outer Radius ( (R_1) )</td>
<td>6 mm</td>
</tr>
<tr>
<td>Radius Ratio</td>
<td>2</td>
</tr>
<tr>
<td>Bottom Angle</td>
<td>20°</td>
</tr>
</tbody>
</table>

Flow measurements were carried out over a range of temperatures comprised from 25°C to 45°C. The temperature was controlled with a circulating bath. The steady-shear viscosity was determined at shear rates ranging from 0.001 up to 10 s\(^{-1}\) at intervals of 10 points per decade.

b) The Bohlin CVO HR 120 rheometer is a stress-imposed rheometer with interchangeable test geometries (can be used with cone-plate, plate-plate, and concentric cylinder geometries). It measures deformation and normal forces over a wide range of both steady and dynamic shear.
Flow measurements were carried out over a range of temperatures comprised from 25°C to 45°C. For temperature control, fluid from a controlled temperature bath was circulated immediately below the flat surface (for cone-and-plate geometry) or the cylinder (for concentric cylinder geometry) onto which the sample was placed.

For the determination of the steady-shear viscosity and normal forces with samples of the chitosan solution we used the cone-and-plate geometry. The solution was placed in the gap between the cone and plate of the Bohlin rheometer. The technical specifications for the selected geometry were:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plate Diameter</td>
<td>40 mm</td>
</tr>
<tr>
<td>Cone Angle</td>
<td>4°</td>
</tr>
<tr>
<td>Cone Truncation</td>
<td>30 microns</td>
</tr>
<tr>
<td>Cone Thickness</td>
<td>1.2 mm</td>
</tr>
</tbody>
</table>

The steady-shear viscosity and normal forces measurements were determined over shear rates ranging from 0.1 up to 1000 s⁻¹ at intervals of 10 points per decade.

The oscillatory shear measurements were performed within the linear viscoelastic regime, where the storage (G') and loss (G'') moduli are independent of the strain amplitude. For the determination of the complex viscosity and moduli with samples of the chitosan solution we used the concentric cylinder geometry. The solution was placed into the major cylinder of the Bohlin rheometer. The technical specifications for the selected geometry in this study were:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cylinders Length</td>
<td>21 mm</td>
</tr>
<tr>
<td>Inner Radius (R₀)</td>
<td>7 mm</td>
</tr>
<tr>
<td>Outer Radius (R₁)</td>
<td>7.7 mm</td>
</tr>
<tr>
<td>Radius Ratio</td>
<td>1.1</td>
</tr>
<tr>
<td>Bottom Angle</td>
<td>15°</td>
</tr>
</tbody>
</table>

First, oscillatory tests were performed at a constant frequency of 1 rad/s varying the amplitude of strain in order to define the linear viscoelastic domain. The strain had to be high enough to give a recordable response and it must be small enough to remain in the
linear region. The complex viscosity and moduli were determined over frequencies ranging from 0.3 to 100 rad/s at intervals of 10 points per decade.

Standard calibration of the apparatus was performed by carrying out measurements with Newtonian fluids, in order to evaluate the response linearity of these systems under the selected operative conditions.

2.6 Results and Discussion

In polar solvents such as water, the chitosan can form polyions. In general these polyions exhibit rheological properties that are different from non-charged polymers: in concentrated solutions, individual polymer chains interact with each other and these interactions can be affected by the degree of ionization of the polyion and polyion/counter-ions interactions.

In order to evaluate the rheological response of concentrated chitosan solutions, the following variables have been analysed:

- Chitosan concentration (0.5, 1.0, 1.5, and 2.0%).
- Counterion concentration (0 and 0.2M in NaCl solution).
- Temperature (25, 30, 37, and 45°C).
- The nature of the acidic solution used: 1.0% acetic acid, 1.0% lactic acid, and 1.0% hydrochloric acid aqueous solutions.

The following features have been observed independently of the chitosan concentration, counterion concentration, temperature, and acidic solution:

For steady-shear flow tests,
- At low shear rates, the steady-shear viscosity is not shear dependent and the viscosity approaches a constant value, the zero-shear-rate viscosity (\(\eta_0\)).
- The steady-shear viscosity decreases as shear rate increases, and generally is shear dependent.
For oscillatory-shear flow tests,

- At low frequencies, the complex viscosity is not frequency dependent and decreases as frequency increases.
- The storage modulus ($G'$) and loss modulus ($G''$) increase as frequency increases.
- $G''$ is always greater than $G'$ ($\eta' > \eta''$). It is an indication that viscous effects are more important than elastic effects.
- The complex viscosity is found to approach the zero-shear-rate viscosity at low frequencies.

For normal forces tests,

- The primary normal stress difference always increases as shear rate increases.
- The normal forces are obtained only at shear rates greater than 10 1/s.

Examples of these behaviors are shown in figures 2.7 and 2.8. These figures are representative for 2% chitosan solutions in 1% acetic acid at 25°C. The behavior showed in figure 2.7 corresponds to classical "shear-thinning" materials where the viscosity decreases with increasing shear rate. Almost all polymer solution and melts that exhibit a shear-rate dependent viscosity are shear-thinning materials [BIRD et al., 1987]. The dependence of the primary normal stress difference on the shear rate can be described by a power-law function with exponent decreasing with shear rate.

The oscillatory-shear flow tests in figure 2.8 show a dominant viscous character of the fluid (the energy dissipation that occurs in the flow) instead of an elastic character of the fluid (the energy storage that takes place during deformation). This behavior occurs very often in dilute and concentrated linear polymer solutions, like polysaccharide solutions [LAPASIN, 1995].

2.6.1 Temperature Effect

Steady-shear flow and oscillatory-shear flow tests were performed at four different temperatures: 25, 30, 37, and 45°C. 25°C was selected as room temperature, 37°C which is the body temperature, and 45°C as the limit temperature to avoid thermal degradation and solvent evaporation. We selected the 30°C point to complete our four-temperatures study.
Figure 2.7 Steady-shear viscosity and first normal stress difference for a 2% chitosan solution in 1% acetic acid at 25°C. [Chitosan: $M_w = 1.08 \times 10^6$, DA = 25%]

Figure 2.8 Storage modulus ($G'$), loss modulus ($G''$), and complex viscosity for a 2% chitosan solution in 1% acetic acid at 25°C. [Chitosan: $M_w = 1.08 \times 10^6$, DA = 25%]
The following features are observed when the temperature is increased:

For steady-shear tests,
- Increasing temperature leads to an increase of the shear rate range where the solution behaves as a Newtonian fluid.
- Increasing temperature causes a decrease of the zero-shear-rate viscosity.
- At high shear rates, a non-Newtonian region is observed which is almost independent of the temperature.
- The values of the primary normal stress difference decrease when temperature increases.

For oscillatory-shear tests,
- The storage modulus ($G'$) and the loss modulus ($G''$) decrease when the temperature increases.

A typical flow curve of the steady-shear viscosity and normal force versus shear rate at various temperatures for 2% chitosan solution in 1% lactic acid is shown in figure 2.9. An example of the temperature effect on the loss moduli ($G''$) for the same system is shown in figure 2.10.

Figure 2.11 shows the effect of temperature on viscosity at constant shear rate. For 2% chitosan solutions dissolved in different acidic solutions we present the temperature/steady-shear viscosity relationship at two different shear rates: 0.12 s$^{-1}$ and 124.0 s$^{-1}$. These shear rates were selected because 0.12 s$^{-1}$ represents a point in the Newtonian region, and 124.0 s$^{-1}$ represents a point in the non-Newtonian region as showed in figure 2.7.

At low shear rates (0.12 s$^{-1}$) the viscosities are very different with respect to the temperature and correspond to the differences between the zero-shear-rate viscosities. At high shear rates (124.0 s$^{-1}$) the viscosities are almost independent of temperature. These behaviors can be explained by the effect of an increase of the chitosan solubility at higher temperatures, which changes the system to a more "Newtonian" flow behavior as demonstrated in figure 2.11. These flow curves are independent of chitosan concentration, counterion concentration, and acidic solution used.
2.6.2 Concentration Effect

The tests were performed at four different chitosan concentrations: 2.0, 1.5, 1.0, and 0.5%. This concentration range was selected because it includes solutions concentrated enough to be considered as “concentrated solutions” (0.5%) and includes the maximum possible concentration for a perfect chitosan dissolution with the acidic solutions used (2.0%).

Figure 2.12 shows examples of the concentration effect on the steady-shear viscosity of chitosan solutions in 1.0% hydrochloric acid at 25°C. We can observe that for high chitosan concentrations, the steady-shear viscosity shows two regions: a first region at low shear where the material has Newtonian behavior, and a second region where increasing the shear rate causes the chitosan solutions viscosity to decrease. Decreasing chitosan concentration resulted in a viscosity less dependent on shear rate.
This behavior can be observed also in figure 2.13, for the steady-shear viscosity at 25°C and constant shear rate (0.12 s⁻¹) versus chitosan concentration (same system as figure 2.12). The steady-shear viscosity of the solutions is increasing faster than the concentration. This is caused by increased interactions between chitosan molecules leading to their entanglements. As the chitosan concentration increases, intermolecular interaction starts to appear and a higher resistance to flow appears in the non-Newtonian region showed in figure 2.12.

Figure 2.14 shows the concentration effect in the complex properties of the chitosan solution. The graphics present the relationship between the complex moduli (G' and G'') and the frequency at different chitosan concentrations (2.0, 1.5 and 1.0%) at 25°C. It can be seen how, for a given frequency, the moduli also increase as the concentration increases. Chitosan solutions at 0.5% in concentration are very diluted solutions and it was not possible to obtain results for their viscoelastic properties due to the sensibility of the rheometer.

2.6.3 Effect of the Acid Used for Chitosan Dissolution

The dissolution of chitosan in acidic aqueous solution is function of the type of acid used [RINAUDO et al., 1999a; RINAUDO et al., 1999b]. In this work chitosan dissolution was performed using three different acidic solutions, all of them being 1.0% acidic aqueous solution. The acids used are acetic acid (CH₃COOH), lactic acid (CH₃CH(OH)COOH), and hydrochloric acid (HCl). Their chemical structures are shown in figure 2.15.

For the different acids used, the reactions taking place are:

- **Chitosan-Acetic acid system**
  \[
  \text{CH}_3\text{CO(OH)} + \text{H}_2\text{O} \leftrightarrow \text{CH}_3\text{COO}^- + \text{H}_3\text{O}^+ \quad (2.19)
  \]
  \[
  \text{Chit} - \text{NH}_2 + \text{H}_3\text{O}^+ \leftrightarrow \text{Chit} - \text{NH}_3^+ + \text{H}_2\text{O} \quad (2.20)
  \]

- **Chitosan-Lactic acid system**
  \[
  \text{CH}_3\text{CH(OH)COOH} + \text{H}_2\text{O} \leftrightarrow \text{CH}_3\text{CH(OH)COO}^- + \text{H}_3\text{O}^+ \quad (2.21)
  \]
  \[
  \text{Chit} - \text{NH}_2 + \text{H}_3\text{O}^+ \leftrightarrow \text{Chit} - \text{NH}_3^+ + \text{H}_2\text{O} \quad (2.22)
  \]
Figure 2.12 Steady-shear viscosity for a chitosan solution dissolved in 1% hydrochloric acid aqueous solution. [Chitosan: \(M_w = 1.08 \times 10^6\), DA = 25%]

Figure 2.13 Steady-shear viscosity (shear rate 0.12 s\(^{-1}\)) at 25°C versus chitosan concentration for solutions dissolved in different acidic solutions. [Chitosan: \(M_w = 1.08 \times 10^6\), DA = 25%]
Figure 2.14 Storage modulus ($G'$) and loss modulus ($G''$) for a chitosan solution dissolved in 1% aqueous hydrochloric acid at 25°C. [Chitosan: $M_w = 1.08 \times 10^6$, DA = 25%]

- Chitosan-Hydrochloric acid system

$$\text{HCl} + \text{H}_2\text{O} \rightarrow \text{Cl}^- + \text{H}_3\text{O}^+ \quad (2.23)$$

$$\text{Chit} - \text{NH}_2 + \text{H}_3\text{O}^+ \leftrightarrow \text{Chit} - \text{NH}_3^+ + \text{H}_2\text{O} \quad (2.24)$$

Figures 2.16 and 2.17 show how the acidic solutions used affect the relationship between steady-shear viscosity versus shear rate and the relationship between complex moduli versus frequency. The systems shown are 2.0% chitosan solutions dissolved in different 1.0% acidic solutions at 25°C.

From these plots we can observe differences in the rheological behavior between a strong acid (hydrochloric acid) and a weak acid (acetic acid or lactic acid). Steady and dynamic tests show that rheological properties of chitosan dissolved in hydrochloric acid are lower in comparison to the other two acids.
Figure 2.15 Molecular structures of the acids used for chitosan dissolution.
Figure 2.16 Steady-shear viscosity for a 2% chitosan solution in different 1% acidic solutions at 25°C. [Chitosan: $M_w = 1.08 \times 10^6$, DA = 25%]

Figure 2.17 Storage modulus ($G'$) and loss modulus ($G''$) for a 2% chitosan solution in different 1% acidic solutions at 25°C. [Chitosan: $M_w = 1.08 \times 10^6$, DA = 25%]

38
It can be stated that:

- The steady-shear viscosity for chitosan solutions dissolved using weak acids are similar for all shear rates.
- The steady-shear viscosity at low shear rate (Newtonian region) is higher for systems in weak acid solutions than for systems in hydrochloric acid. At high shear rates (non-Newtonian region) the behaviors are almost similar.
- For the complex moduli, the values are also similar for weak acids. They are lower for the solution in hydrochloric acid, indicating a strong interaction between the chitosan and the acid.

In figure 2.11 we observe a temperature effect over the acidic solution used at a 2% chitosan concentration. For both shear rates presented (0.12 and 124.0) and for all the temperature range (25 to 45°C) the steady-shear viscosity is lower for the chitosan solution in a hydrochloric acid medium. The same behavior can be observed in figure 2.12, when we plotted the relationship between the steady-shear viscosity and the chitosan concentration in solution at 25°C. For both shear rates (0.12 and 124.0) and at all chitosan concentrations (0.5 to 2%), the viscosity is higher for solutions in acetic acid or lactic acid.

This behavior can be explained by [CHEN et al., 1994]:

- Steric effects exerted by anions.
- Different neutralisation effect of the protonated amino group.
- Intramolecular electrostatic repulsion effect exerted by the weak acid ions.
- Differences in surface tension of the solutions.

In regard to the steric effect, it is easy to see that acetate and lactate ions are bulkier than chloride ions. The dissociation constants (\(K_a\)) in aqueous solution of hydrochloric acid and the weak acids studied are very different. This constant is the equilibrium constant for the acid dissociation in water and corresponds to:

\[
HA_{(eq)} + H_2O_{(l)} \leftrightarrow H_3O^{+}_{(eq)} + A^{-}_{(eq)}
\]  

(2.25)

\[
K_a = \frac{[H^+][A^-]}{[HA]}
\]  

(2.26)
This constant is greater than 1.0 for hydrochloric acid (HCl concentration is so small that it is difficult to measure it with exactitude, but $K_a = 1$ is a very good value for this strong acid), 1.75 x 10$^{-5}$ for acetic acid, and 1.37 x 10$^{-4}$ for lactic acid. The values were measured in aqueous solution at 25°C [ZUMDAHL, 1998]. We can observe that under similar conditions there are more chloride ions than acetate or lactate acid ions (weak acid), helping to the neutralisation effect.

Table 2.3 pH's in chitosan solutions.

<table>
<thead>
<tr>
<th>1.0% Acidic Solution</th>
<th>NaCl Solution</th>
<th>Chitosan Concentration (%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic Acid</td>
<td>0 M</td>
<td>2.0</td>
<td>4.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>4.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>4.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>3.66</td>
</tr>
<tr>
<td></td>
<td>0.2 M</td>
<td>2.0</td>
<td>4.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>4.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>4.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>3.73</td>
</tr>
<tr>
<td>Lactic Acid</td>
<td>0 M</td>
<td>2.0</td>
<td>5.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>4.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>3.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>3.08</td>
</tr>
<tr>
<td></td>
<td>0.2 M</td>
<td>2.0</td>
<td>5.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>4.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>3.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>3.14</td>
</tr>
<tr>
<td>Hydrochloric Acid</td>
<td>0 M</td>
<td>2.0</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>0.2 M</td>
<td>2.0</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>0.60</td>
</tr>
</tbody>
</table>
The intramolecular electrostatic repulsion exerted principally by the weak acid ions makes the molecule unfold by increasing the charge density (protonated amino group) on the backbone of the chitosan that increases the hydrodynamic volume of the molecule and thus the viscosity [CHEN et al., 1994].

An increase of surface tension causes different solute-solvent interactions. These interactions affect the hydrodynamic volume, the shape of the solute, and its solubility. The presence of the chloride ion has a larger surface tension increment than weaker acid ions [CHEN et al., 1994].

Varying pH, ionic strength, concentration, time, or the nature of the media can change the electric charge density on the molecule. These changes will affect the solutions conformation [CHEN et al., 1994]. However, the combined effect of these variables needs further exploration, especially the combined effect of pH and type of anion. In this study the pH of the solutions was not controlled. The solutions were prepared as function of the desired chitosan concentration, using always 1.0% acidic solutions. Nevertheless, we measured the pH's of the solutions (Table 2.3) and some observations can be made.

Higher pH values correspond to systems that were dissolved in weak acid solutions. They are systems that showed higher shear-viscosity values. On the other hand, very low pH values were obtained for chitosan-HCl systems. In the case of low pH values (HCl solutions), the effect of an excess number of ions on the shear-viscosity of chitosan systems indicated that the conditions studied were close to that of complete suppression of electrostatic repulsion by ion (chloride ions).

In general, the stronger the acid solution, the lower the viscosity. This is believed to be caused by the screening effect of the electrostatic force (similar to the effect of adding salt) of the anionic group in solution. This effect increases with increasing strength of the acid or decreasing pH value of the solvent [WANG et al., 1994a].
2.6.4 Effect of the Counterion Concentration

Generally the viscosity of charged polysaccharides in solution decreases with the presence of a salt as a result of the physical entanglement of polysaccharide molecules [Kienzle-Sterzer, 1984]. In order to study the effect of the counterion presence (0.2M sodium chloride solution) upon the rheological response of concentrated chitosan solutions, steady and dynamic tests were performed with different chitosan solutions. As previously done, the tests were performed for the entire range of temperature and chitosan concentrations.

The results obtained are shown in figures 2.18 to 2.20. The effect of the ionic strength on the steady-shear viscosity at 30°C, for all chitosan concentrations used in this study is presented. Figure 2.18 shows this effect when chitosan was dissolved in aqueous acetic acid solution, figure 2.19 for lactic acid, and figure 2.20 for hydrochloric acid.

These figures show that:

- In the cases of chitosan dissolved with acetic acid and lactic acid (figures 2.18 and 2.19) there are differences with respect to the concentration. For 2% chitosan solution the steady-shear viscosity in all the shear rate ranges is almost the same with or without the presence of salt (NaCl solution). When the chitosan concentration begins to decrease, the difference in viscosity between the solution with salt and the solution without salt increases, at 0.5% chitosan concentration the viscosity difference is very significant.

- In the case when hydrochloric acid is used for chitosan dissolution (figure 2.20), there are almost no differences with respect to the concentration in both cases studied: with or without the addition of NaCl.

The same behavior is observed in the dynamic tests. Figures 2.21 to 2.23 show the effect of the counterion presence on the loss modulus (G") at 30°C when the chitosan is dissolved in different acidic solutions, with or without the addition of 0.2M NaCl. These plots present results for three different chitosan concentrations: 2.0, 1.5, 1.0%; the results for 0.5% chitosan concentration are not presented since the complex viscosity and dynamic moduli were below the technical limit of the rheometer used.
Figure 2.18 Effect of the presence of salt on the steady-shear viscosity for a chitosan solution in 1% acetic acid solution at 30°C. [Chitosan: $M_w=1.08\times10^6$, DA = 25%]

Figure 2.19 Effect of the presence of salt on the steady-shear viscosity for a chitosan solution in 1% lactic acid solution at 30°C. [Chitosan: $M_w=1.08\times10^6$, DA = 25%]
Figure 2.20 Effect of the presence of salt on the steady-shear viscosity for a chitosan solution in 1% hydrochloric acid solution at 30°C. [Chitosan: $M_w = 1.08 \times 10^6$, DA = 25%]

Figure 2.21 Effect of presence of salt on the loss modulus ($G''$) for a chitosan solution in 1% acetic acid solution at 30°C. [Chitosan: $M_w = 1.08 \times 10^6$, DA = 25%]
At high concentration (2%) the modulus is the same for the systems with and without salt. However, when the concentration decreases, systems where the chitosan was dissolved using weak acid solutions show differences between the loss modulus (G'') of the solution with and without salt. The solution with the presence of a counterion has a lower modulus than the system without the counterion. There are small differences when the chitosan was dissolved in the strong acid (HCl).

<table>
<thead>
<tr>
<th>Chitosan Concentration (%)</th>
<th>η without salt / η with salt</th>
<th>η without salt / η with salt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chitosan – Acetic acid solution</td>
<td>Chitosan - HCl solution</td>
</tr>
<tr>
<td>2.0</td>
<td>1.16</td>
<td>0.83</td>
</tr>
<tr>
<td>1.5</td>
<td>1.16</td>
<td>1.01</td>
</tr>
<tr>
<td>1.0</td>
<td>1.61</td>
<td>0.91</td>
</tr>
<tr>
<td>0.5</td>
<td>2.90</td>
<td>0.92</td>
</tr>
</tbody>
</table>

The importance of these differences can be viewed in figure 2.24 and table 2.4 where the effect of the salt on the relationship between viscosity and chitosan concentration is shown. The figure and table show the results for the systems chitosan-acetic acid and chitosan-hydrochloric acid at 30°C and a shear rate of 1.25 s⁻¹.

We can observe that the viscosity for the acetic acid (figure 2.24-a) is lower for the salt-system when the concentration decreases. In the case of chitosan dissolved in hydrochloric acid solution (figure 2.24-b) the viscosity remains almost constant after the addition of the salt solution. Table 2.4 shows the relationship between the solution with and without salt for the systems chitosan-acetic acid and chitosan-HCl. For the case of the chitosan-acetic acid system this value grows with the decrease of chitosan concentration, whereas for the chitosan-HCl systems the relationship remains constant.

The presence of a counterion concentration changes the density of the intermolecular physical entanglements in concentrated chitosan solutions. The effect of an excess number of chloride ions on the steady-shear viscosity indicates a complete suppression of electrostatic repulsion [CHEN et al., 1994].

In the case when an excess of chloride ions are present before the salt addition (chitosan dissolved in HCl solutions), the difference in the rheological results of the systems with and
without salt is minimal. The addition of chloride ion does not induce any rheological effect since there is no alteration of the electrostatic fields already well established by the presence of the chloride ion in solution.

![Graphs showing viscosity vs. chitosan concentration](image)

**Figure 2.24** Effect of the presence of salt on the relationship between viscosity and chitosan concentration. (a) For the system chitosan-acetic acid solution, (b) for the system chitosan-hydrochloric acid solution. The temperature is 30°C and shear rate is 1.25 s⁻¹. [Chitosan: M₆₈ = 1.08x10⁶, DA = 25%]

However when the chitosan was dissolved in weak acid solutions (presence of acetate ions or lactate ions) the effect of the addition of chloride counterions is important. When an excess of chloride ion is added, these ions seek NH₃⁺ cations that remain non-neutralised on the chitosan. This process will change the electrostatic field in the solution and will reflect in a viscosity decrease.

At low chitosan concentrations the movement of the chloride ions is easier in the solution. This is why the rheological differences are more notable at low chitosan concentrations. At 2.0% chitosan concentration the medium is so dense that the chitosan conformation, quite stable, makes almost impossible the distribution of the chloride counterions.
3. RHEOLOGICAL MODELING FOR
CHITOSAN SOLUTIONS

3.1 Summary

The rheological modeling of the results presented in the previous chapter is discussed in this chapter. Steady-shear tests were modelled using the Cross viscosity equation, which uses three parameters for describing the relationship between viscosity and shear rate: the zero shear rate viscosity (η₀), a characteristic time (τ), and the power law parameter (n). The Cross model and an empirical relationship called Wagner's relationship are used for normal forces. Wagner's relationship provides a relation that can be used to estimate normal stress differences in shear flow using a strain dependent parameter.

For modelling the oscillatory-shear results we used the generalised Maxwell model. The simplest model for viscoelastic behavior can be obtained by combining a Hooke solid with a Newtonian dashpot; this is the well-known Maxwell model. The generalization is based on the idea that polymer macromolecules are of different length and possibly form a network leading to a distribution of relaxation times.

Steady-shear rheological properties and oscillatory-shear properties of the solutions are related. The steady viscosity function η, can be related to the complex viscosity, η*, and the dynamic viscosity function η'; while the primary normal stress difference N₁, can be related to η''/ω. The first relationship used is the Cox-Merz rule. It is an empirical relationship stating that for small shear rates, η* and η' are approximately equal and of similar magnitude to the steady viscosity η. Another relationship used is Laun's rule that predicts the primary normal stress difference from more readily available linear viscoelastic data.

We present also temperature and concentration superpositions in form of master curves. The superposition principle is based on the idea that concentration and temperature have similar or comparable effects on the relaxation of the systems. The rheological curves have similar shapes at different temperatures and polymer concentrations. This similarity provides the basis of an empirical method, known as the method of reduced variables, for combining data taken at several different temperatures and polymer concentrations into one master curve.
• Bingham plastics: Systems that presents the yield stress, which is the peculiar feature of the plastic behavior. Examples are the margarine, vegetable fats, etc.
• Shear-thinning materials: Systems in which viscosity decreases with shear rate. Examples are protein solutions, creams, concentrated juices, comestibles purees, bronchial secretions, cell protoplasm, synovial fluids, etc.
• Shear-thickening materials: Systems in which viscosity increases with shear rate. Examples are found principally in concentrated suspensions of stabilised particles.

3- Non-Newtonian and time dependent: The time-dependent phenomena (like thixotropy and anti-thixotropy) are often exhibited by dispersed systems in which the disperse phase is made up of particle aggregates and in highly concentrated particle suspensions. Examples are condensed milk, carbohydrate suspensions, blood, lymph, etc.

4- Viscoelastic (liquids and semi-solids): A different time-dependent phenomena in which the response of stresses and strains in the fluid to changes in imposed strains and stresses respectively is not instantaneous; presented independently of the non-Newtonian time dependent systems, for example in weak gels and many dispersed systems.

3.2.1 Steady-Shear Viscosity and Normal Forces Models

For dilute polysaccharide solutions we consider that the shear rate results associated to a constant shear stress do not change as long as the shear rate is maintained; they do not show time-dependent effects. This is not the case for concentrated systems. At a given shear rate the measured shear stress, and hence the viscosity, can either increase or decrease with time of shearing, until a steady value is reached, and such changes are reversible [LAPASIN et al., 1995].

The vast majority of concentrated polymer solutions exhibit shear-thinning behavior in a steady simple shear flow. Somewhat more complex behaviors have been observed for some organic polymer solutions, characterized by the appearance of a shear-thickening region for intermediate values of shear rate [BARNES, 1989]. In general the viscosity varies between two extremes \( \eta_0 \) and \( \eta_\infty \) corresponding to the zero-shear viscosity and the viscosity of the solvent respectively. The difference \( \eta_0 - \eta_\infty \) is a function of the polymer, but varies substantially with concentration, polymer molecular weight, polydispersity, and temperature.
For systems at medium rates of shear, the non-Newtonian viscosity obeys a general power-law relationship as:

\[ \eta = K\dot{\gamma}^{n-1} \]  

(3.1)

One of the main reasons for the popularity of this model is that it is easy to handle in its logarithmic form. A straight line is obtained on a log-log plot of viscosity versus shear rate over more than one decade. K is the consistency factor and n is the flow behavior index varying between 0 and 1.0 for shear-thinning fluids and greater than 1.0 for shear-thickening fluids. Temperature and concentration affect the values of K and n. With the exception of dilute solutions, the power law exponent (n-1) gradually increases with increasing concentration, approaching a constant value for sufficiently high concentration values. Dail and Steffe [1990a] said that n is the most important factor determining the flow rate of a product, since small changes in n cause larger fluctuations in the flow rate than similar changes in K.

Some examples of this type of behavior are 1-10% starches [GRISKEY et al., 1971; DAIL et al., 1990a; DAIL et al., 1990b], 0.5-2.0% guar gums and other hydrocolloids [BALMACEDA et al., 1973]. Holdsworth [1971] and Steffe [1984] compiled magnitudes of the power law parameters reported in the literature, and these references should be consulted for data on specific biological fluids. Some other specific findings of the models can be derived directly from the examination of the functional dependence and the predicted profiles of the flow curves. Here we present the most frequently used models:

a) Bingham Model [BINGHAM, 1916]

\[ \tau = \tau_0 + K\dot{\gamma} \]  

(3.2)

Many types of biological solutions exhibit a yield stress (\(\tau_0\)) and are said to show plastic or viscoplastic behavior. The yield stress is defined as the stress below which no flow can be observed under the conditions of experimentation. This model is very popular in the food industry. For example it was applied to mustard [OFOLI et al., 1987] and meat extracts [HALMOS et al., 1981].
The Heinz-Casson model has been used with 5.5-7.3% gelatinised starch suspensions [DOLAN et al., 1990] and for protein dough extrusion [MORGAN et al., 1989]. The generalised Casson model has been applied to 0.5-1.2% xanthan and 0.5-2.0% guar gum [RAO et al., 1975].

d) Cross Model [CROSS, 1965]

\[
\frac{\eta - \eta_\infty}{\eta_0 - \eta_\infty} = \frac{1}{\left(1 + \frac{\gamma\eta_\infty}{\eta_0 \gamma_\infty}ight)}
\]  

(3.7)

This model has been used for starch flow properties [HÄRRÖD, 1989] and for guar gum solutions [DOUBLIER et al., 1981], who showed that the model was applicable over a range of five decades in shear rates.

The two-parameter Cross model (\(\eta_\infty = 0, m = 2/3\)) has been used with different polysaccharides solutions: hydroxyethyl guar gum, carboxymethyl-cellulose, and sodium alginates solutions [LAPASIN et al., 1988].

e) Tiu and Boger Model [TIU et al., 1974]

\[
\tau = \lambda_s\left[\tau_0 + K\dot{\gamma}^n\right]
\]  

(3.8)

This model is based on a modified Herschel-Bulkley model using a time-dependent structural parameter \(\lambda_s\), where \(\lambda_s < 1\) at \(\dot{\gamma} = 0\), and \(\tau_0\) is the yield stress. This model and other more complex forms of the Tiu and Boger model have been applied to mayonnaise [TIU et al., 1974; TIU et al., 1982].

f) Powell-Eyring Model [REE et al., 1958]

\[
\tau = \eta_\infty\dot{\gamma} + B\sinh^{-1}(\beta\dot{\gamma})
\]  

(3.9)

This model is based on the theory of rate processes and structural parameters. The coefficient \(\beta\) is the relaxation time, and \(\eta_\infty\) is the viscosity at infinite shear rate. The model has
been extensively applied to guar gum polysaccharide products [DOUBLIER et al., 1976; DOUBLIER et al., 1981].

g) Carreau Model [CARREAU, 1972]

\[ \frac{\eta - \eta_\infty}{\eta_0 - \eta_\infty} = \left[ 1 + \left( \lambda \dot{\gamma} \right)^2 \right]^{\frac{\eta_0 - 1}{\eta_0 - \eta_\infty}} \]  

(3.10)

This model is applicable to both low and high shear rates. \( \lambda \) is a characteristic time parameter with dimensions of time, \( \eta_0 \) and \( \eta_\infty \) are the viscosities at \( \dot{\gamma} = 0 \) and \( \dot{\gamma} = \infty \) respectively. The model has been applied successfully to mayonnaise [TIU et al., 1982].

3.2.1.1 Polymer Concentration Effect

In the absence of structure-induced formation the rheological behavior of concentrated polysaccharide solutions is monotonically shear-thinning which substantially increases with polymer concentration. Typical results are obtained from aqueous dextran solutions. At any given concentration, \( \eta_0 \) can be determined and its value increases with concentration as \( \text{C}^{2.2} \) [SABATIÉ et al., 1988]. Xanthan gum solutions [MILAS et al., 1990] show similar behavior for which the viscosity in the concentrated domain increases as \( \text{C}^{3.7} \). This concentration dependence is typical of flexible random coil polymers.

There are two types of models that are used to relate the rheological properties to the polymer concentration: Empirical models and molecular models.

a) Empirical Models:

There are several empirical equations than have been used with biological fluids. For example linear models:

\[ \eta = A + BC \]  

(3.11)

A and B are constants and C is the polymer molar concentration. This equation was applied to cow's and soy milk solutions [OGUNTUNDE et al., 1991].
There are power law models such as:

\[ \eta = AC^B \]  
(3.12)

\[ \eta = A_1C_1^{A_2}C_2^B \]  
(3.13)

Where in equation 3.12 A and B are constants and in equation 3.13 the model has two concentration parameters (useful for multicomponents solutions or dispersions). Speers and Tung [1986] used power law models with 0.05-1.0% xanthan gum solutions and Launay et al. [1983] applied it to 5.0-10.0% starch pastes.

Finally, there are exponential models, such as:

\[ \eta = Ae^{BC} \]  
(3.14)

where A and B are constants. Some examples are gums and hydrocolloids [RAO et al., 1975] and juice concentrates [RAO et al., 1981; RAO, 1982].

b) Molecular Models
The most important molecular model that relates viscosity to polymer concentration is the Huggin’s model. Viscosity-concentration relationships based on the interaction between polymeric molecules and solvent have proven to be highly successful for correlating the two factors in the case of dilute solutions. The Huggin's relationship takes the form [HOLDSWORTH, 1993]:

\[ \eta_p = \eta + k[\eta]^2C \]  
(3.15)

where

\[ \eta_p = \left( \frac{\eta_p}{\eta_s} \right) - 1 \]  
(3.16)
\( \eta_{sp} \) is the specific viscosity based on \( \eta_s \), the viscosity of the solution, and \( \eta_s \) the viscosity of pure solvent. \([\eta]\) is the intrinsic viscosity of the solution and is obtained from the specific viscosity extrapolated to zero concentration. The model has been used, for example, to interpret the flow behavior of soy protein fractions [DIEP et al., 1982] and chitosan solutions [MAGHAMI et al., 1988; WANG et al., 1991; KASAAI et al., 2000a].

There is another molecular model that has been used in polysaccharide solutions: the Fuoss model [ELFAK et al., 1978]:

\[
\frac{\eta_{sp}}{C} = \frac{A}{1 + [\eta]\sqrt{C}}
\]  

(3.17)

The model has been used to interpret the behavior of sodium carboxymethyl cellulose and \( \kappa \)-carrageenan in polysaccharide solutions. It predicts the reduction in intrinsic viscosity of the solutions due, primarily, to competitive hydration which is experienced by neutral molecules and to the interactions of the polyanions in the polysaccharides [ELFAK et al., 1978].

3.2.1.2 Polymer Molecular Weight Effect

This is another parameter that has a strong influence on the shear-dependent behavior. For most of the biological solutions, as molecular weight increases the non-Newtonian effects are shifted to lower shear rates. We can observe a stronger power-law dependence of \( \eta_0 \) on molecular weight with respect to that observed in the dilute regime.

For longer chains the form of the structure factor changes, becoming a much stronger function of molecular weight. At high molecular weights the well known 3.4-power dependence results:

\[
\eta_0 = KM_w \quad M_w < M_c
\]  

(3.18)

\[
\eta_0 = K'M_w^{3.4} \quad M_w > M_c
\]  

(3.19)
The change in molecular weight dependence occurs smoothly but over a relatively narrow range in molecular weight, at some critical molecular weight $M_c$ [GRAESSLEY, 1974].

There are lots of works in polysaccharide solutions in which a relationship between the polymer concentration and the viscosity is determined. For example: Gravanis [1990] compared the flow curves of 0.1% succinoglycan salted aqueous solutions with different polymer fractions. Milas [1990] found that the dependence of the specific viscosity in concentrated xanthan solutions becomes $C^{3.7}M_w^{-4}$. Mucha [1997] studied chitosan solutions, the concentration dependence of the viscosity was proportional to $M_w^{1.8}$. The relationship between polymer concentration and viscosity is used to describe the behavior of the polymer in solution.

Polydispersity is an important factor in rheological behavior and can have a significant influence on the flow curve of non-Newtonian fluids. In general polydispersity acts in such a way as to broaden the flow curves, making them more flat and shifting the departure from $\eta_0$ to lower shear rates [CROSS, 1969].

3.2.1.3 Temperature Effect

Increasing temperature leads to a decrease of $\eta_0$ and an increase of the critical shear rate of the non-Newtonian onset. This implies that the influence of the temperature on viscosity progressively decreases as the shear rate increases. In general, the effect of temperature on the viscosity or apparent viscosity determined at a specific shear rate can be expressed by the Arrhenius relationship:

\[
\eta(\dot{\gamma}) = A e^{\frac{E(\dot{\gamma})}{RT}}
\]  

(3.20)

The activation energy $E(\dot{\gamma})$ reaches a maximum in the Newtonian region and substantially decreases with increasing shear rate, being strictly connected with the decrement in entanglement density. The value of $E(\dot{\gamma})$ for any given polymer solution is generally higher than that of the corresponding pure solvent, and is a power law function of the polymer concentration $C$. Studies on the effect of temperature over the rheological properties in
solutions can confirm the occurrence of conformational transitions [ROCHEFORT et al., 1987]. Magnitudes of activation energy have been tabulated for a number of biological fluids, like raspberry juice (75.3 kJ/mol), banana juice (25.07 kJ/mol), tomato concentrated (9.3 kJ/mol), apricot purée (1.8 kJ/mol), pear purée (1.74 kJ/mol), etc. [HOLDSWORTH, 1971 and 1993; RAO, 1977]. There are also empirical models that are less frequently used but often cover a wider range of temperatures, for example:

i) Fulcher Model

\[ \ln \eta = \frac{B}{T - T_0} \]  \hspace{1cm} (3.21)

which has been applied to sugar solutions and fruit juices [RAO et al., 1986].

ii) Power and Exponential Models

\[ \eta = AT^B \]  \hspace{1cm} (3.22)

and

\[ \eta = \exp \left( C + \frac{D}{T} \right) \]  \hspace{1cm} (3.23)

where A, B, C, and D are functions of concentration. These models have been used for 10-65% orange juice [MORESI et al., 1980].

3.2.1.4 Models for Thixotropic Solutions

Thixotropic behavior has been found in various works. In the case of thixotropy, we observe a decrease of viscosity with time, when the sample is subject to a constant rate of shear. This decrease in viscosity is associated with a change in structure, as a result of the applied shear rate; a time-independent, steady shear viscosity is eventually reached.

Higgs and Norrington [1971] studied the thixotropic behavior of sweetened condensed milk. They measured the coefficient of thixotropic breakdown with time, B, which indicates the rate
of breakdown with time at constant shear rate, and the coefficient of thixotropic breakdown due to the increasing shear rate, M, which indicates the loss in shear stress per unit increase in shear rate. The coefficients were estimated from the equations:

\[ B = \frac{\eta_1 \eta_2}{\ln(t_2 - t_1)} \]  \hspace{1cm} (3.24)

and

\[ M = \frac{\eta_1 \eta_2}{\ln(N_2 - N_1)} \]  \hspace{1cm} (3.25)

In equation 3.24, \( \eta_1 \) and \( \eta_2 \) are viscosities measured after times \( t_1 \) and \( t_2 \) respectively. In equation 3.25, they are measured at angular speeds \( N_1 \) and \( N_2 \) respectively.

Tiu and Boger [1974] employed a kinetic rheological model to characterize the thixotropic behavior of mayonnaise samples. It was based on the Herschel-Bulkley model multiplied by a structural parameter, \( \lambda \) which ranges between an initial value of unity for zero shear time to an equilibrium value \( \lambda_e \), which is less than unity.

\[ \tau = \lambda (\tau_0 + K\dot{\gamma}^n) \]  \hspace{1cm} (3.26)

The decay of the structural parameter was assumed to obey the second order rate equation

\[ \frac{d\lambda}{dt} = -K_1(\lambda - \lambda_e)^2 \]  \hspace{1cm} (3.27)

\( K_1 \) is a parameter that is a function of the shear rate. If all the parameters are determined from experimental data, equations 3.26 and 3.27 can be used to complete the rheological characterisation of a thixotropic fluid.
3.2.1.5 Normal Stress

The available experimental information on normal stress in concentrated polymer solutions is quite limited, particularly for polysaccharides. We know that normal stresses are increasing functions of the shear rate, and their dependence on shear rate is stronger than the corresponding shear stress, so that their absolute values cannot be easily measured at low shear rate.

There are very few normal stress data relative to concentrated polysaccharide solutions. The first normal stress difference \( N_1 \) is a power-law function of shear rate, with a power law index around 1.0 for dilute xanthan solutions [WHITCOMB et al., 1978], and dextran [SABATIÉ et al., 1986], whereas it ranges from 1.6 to 1.8 for solutions of extracted and commercial xanthan gum in the presence and absence of salt [BEWERSDORFF et al., 1988].

\( N_1 \) values for 3\% w/w xanthan gum solution in water were measured by Jones [1987]. They found it to depend on \( \dot{\gamma}^{0.58} \). Gupta [1986] obtained \( N_1 \) data for a solution of 0.06\% w/w xanthan gum in a solvent of corn syrup and water; the \( N_1 \) values were shown to be proportional to the shear rate with an exponent around 0.75.

For aqueous solutions of guar gum at lower concentrations (<0.5\% w/w) \( N_1 \) is directly proportional to shear rate [WHITCOMB et al., 1980]. Increasing the concentration decreases the shear dependence of \( N_1 \). This shear-dependence is lower than expected, since all the molecular and continuum models applicable to polymer solutions predict that \( N_1 \) is, at low shear rates, a quadratic function of the shear rate.

Dickie and Kokini [1982] employed a power relationship to describe the effect of shear rate on the primary normal stress difference:

\[
N_1 = m' \dot{\gamma}^{n'}
\]  \hspace{1cm} (3.28)

The magnitudes of \( m' \) and \( n' \) for selected solutions are presented by Rao [1985].
Zirnsak [1999] supposed that the dumbbell model is applicable to xanthan solutions systems. They used it in the limit of very large shear rates and found a power-law limiting expression [STEWARD et al., 1972]:

\[ N_1 = 1.20 \frac{CRT}{M_w} (\lambda_D \dot{\gamma})^{\gamma_s} \]  \hspace{1cm} (3.29)

C = concentration (w/w)
R = gas constant
T = absolute temperature
M_w = molecular weight

and \( \lambda_D \) is the time constant for the rigid dumbbell that can be determined from the zero-shear rate viscosity according to:

\[ \lambda_D = \frac{(\eta_0 - \eta_s)M_w}{CRT} \]  \hspace{1cm} (3.30)

\( \eta_0 \) = zero-shear rate viscosity
\( \eta_s \) = solvent viscosity

They used this model for \( N_1 \) predictions of dilute solutions of rigid rod-like molecules and demonstrated a good fit to the experimental data.

3.2.2 Time-Dependent Models

The time-dependent properties manifest themselves as a consequence of changes in flow conditions or in the force field imposed on the fluid. They are more or less pronounced depending on the characteristic time of the material and the flow process. In the dilute regime, the characteristic times of the solution are very short and close to that of the solvent. This implies that the time-dependent properties cannot be detected easily. This is no the case for concentrated polymer solutions, for which some general features of the time-dependent properties can be derived.
The viscosity of many food products depends not only on the shear rate but also on the time of shearing. These materials are said to be thixotropic if the shear stress decreases with time and rheoplectic if the shear stress increases with time. The most used time-dependent models in biological fluids are:

a) Weltmann Model [WELTMANN, 1943]

\[ \tau = A_1 + B_1 \log(t) \]  \hspace{1cm} (3.31)

The Weltmann model is the simplest model that has been developed to characterise thixotropic products. The coefficient \( A_1 \) is known as the shear coefficient, and \( B_1 \) is the time coefficient of thixotropic behavior. The model has been used for a range of thixotropic products, for example with pea pastes [BATTACHARYA et al., 1992].

b) Hahn Model [HAHN et al., 1959]

\[ \log(\tau - \tau_e) = A_2 - B_2 t \]  \hspace{1cm} (3.32)

This model was developed on the basis of a structural molecular model that involved the equilibrium between entanglement and disentanglement of long chain molecules. \( \tau_e \) is the equilibrium shear stress. The model has been used with 14.7\% apricot purée [DURAN et al., 1985].

c) Oldroyd-B Model [OLDROYD, 1953]

\[ \frac{\tau(t)}{\tau_e(\dot{\gamma}_e)} = \frac{1-(A_1/A_2)}{1+(A_1/\dot{\gamma}_e)2}\exp\left(-\frac{t}{\lambda_1}\right) \]  \hspace{1cm} (3.33)

The Oldroyd-B or convected Jeffreys model predicts a simple exponential decay for the shear stress, after the stress has achieved its equilibrium value \( \tau_e(\dot{\gamma}_e) \) during steady shear at constant shear rate \( \dot{\gamma}_e \). Liang and Mackay [1993] described stress jumps experimentally observed for xanthan solutions.
3.2.3 Viscoelastic Models

Experimental tests under oscillatory shear conditions are a consistent part of a common rheological characterisation of concentrated polymer solutions. Data obtained from dynamic tests are inherently conceived for the analysis of the viscoelastic properties of materials; at the same time, they represent a fundamental challenge for testing viscoelastic models.

3.2.3.1 The Maxwell Models

The first attempt to obtain a viscoelastic constitutive equation appears to have been that of Maxwell who developed a theory for viscoelasticity, because he thought that gases might be viscoelastic [BIRD et al., 1987]. One way to generalise the Maxwell model is to construct a superposition of Maxwell models.

\[ \eta'(\omega) = \sum_{k=1}^{\infty} \frac{\eta_k}{1 + (\lambda_k \omega)^2} \]  \hspace{1cm} (3.34)

\[ \frac{\eta''(\omega)}{\omega} = \sum_{k=1}^{\infty} \frac{\eta_k \lambda_k}{1 + (\lambda_k \omega)^2} \]  \hspace{1cm} (3.35)

Thurston and Pope [1981] used a generalised model containing N Maxwell elements (N relaxation processes plus a non-relaxing dash pot element) in order to investigate the viscoelastic properties of xanthan solutions. With 3 discrete relaxation processes they were able to describe how viscoelasticity changes with both shear rate and frequency and how the steady flow viscosity changes with shear rate.

3.2.3.2 Rouse and Zimm Models

This type of models predicts the reduced moduli and relaxation time of flexible random coil molecules. At high frequencies the reduced moduli of the Rouse theory become equal and increase together with a slope of \( \frac{1}{2} \), while those in the Zimm theory remain unequal and increase in a parallel manner with a slope of \( \frac{3}{4} \). The theories of Rouse [1953] and Zimm [1956] have been used to study the conformation of apple, tomato, and citrus pectins in solution [KOKINI, 1994]. The experimental values for these systems follow the expected
values for Zimm type behavior relatively well with the limiting slope of the reduced moduli closer to the calculated slopes of 2/3 for the Zimm model than that of 1/3 for the Rouse model.

The storage ($G'$) and loss ($G''$) moduli for dilute xanthan solutions agree with the predictions of the high frequency limit of Rouse and Zimm models [ZIRNSAK et al., 1999]. As the solvent viscosity increases, at a fixed concentration, $G'$ and $G''$ data move from being Rouse-like to Zimm-like, due possibly to an increase in the hydrodynamic interaction with increasing solvent viscosity. Tam and Tiu [1989] obtained the same results.

3.2.4 Constitutive Models

Constitutive equations enable the simulation of a wide range of rheological data obtained by a variety of experiments. These models necessitate rheological constants that are determined either from molecular properties or from an independent set of experiments. Constitutive models aim at relating rheological properties to molecular architecture and to the prediction of all components of stress generated as a result of a given deformation history. In order to achieve this goal, idealisation of molecular architecture of conformation is necessary. Such idealisation lead to the molecular theories of rheology.

Molecular theories started by considering dilute solutions of high molecular weight polymeric materials. These theories are particularly useful in characterising the effect of long range conformation on flexibility of carbohydrates as well as proteins. A typical idealisation is the use of the freely jointed chain consisting of springs and beads [BIRD et al., 1987].

3.2.4.1 The Bird-Carreau Model

Dilute solution molecular theories have evolved to predict also rheological properties of concentrated polymer systems. Some constitutive theories are based on principles of continuum mechanics such as the Bird-Carreau model [BIRD et al., 1968] and are semi-empirical in nature. Such models incorporate key assumptions pertaining to network formation and dissolution that clearly occur during deformation processes.

The Bird-Carreau model involves taking an integral over the entire deformation history of the material. It is based on the Carreau constitutive theory of molecular networks which is able to
explain viscoelastic behavior by assuming that deformation creates and destroys temporary crosslinks [BIRD et al., 1968].

3.2.4.2 The Doi-Edwards Model

Other models have a molecular and conformational basis and enable us to predict rheological properties from detailed understanding of molecular structure. An example is the Doi-Edwards constitutive model [DOI et al., 1978a, b, c].

The Doi-Edwards theory of viscoelasticity is explained by considering entanglements within the polymer network. It is based on the reptation theory. A model chain is constructed to describe molecular motions in densely populated system using appropriate assumptions. For example, each polymer chain moves independently in the average field imposed by the other chains. A three-dimensional cage represents the average field. In this cage each polymer is confined in a tube-like region surrounding it. The primitive chain can move randomly forward or backward only along itself.

3.3 Rheological Models for Chitosan Description

In this section we describe the rheological models and relationships used for the modelization of the experimental results presented in chapter 2.

The models and relationships used are:

- For steady-shear: the Cross model.
- Normal forces are correlated using Wagner’s relationship and Cross model.
- For oscillatory-shear tests: the generalised Maxwell model with 6 or 4 parameters.
- Relations between linear viscoelastic properties and viscometric functions are obtained using the Cox-Merz rule and Laun’s rule.
- For steady-shear, normal forces, and oscillatory-shear tests master curves are obtained combining data taken at several temperatures and concentrations using the time-temperature superposition principle.
- For steady-shear test, an Arrhenius type of equation is used in order to evaluate the activation energy for flow.
3.3.1 Cross Model [CROSS, 1965]

The largest and probably most important class of non-Newtonian fluids are those which exhibit pseudoplastic (shear-thinning) flow (which show a decrease in viscosity with increasing rate of shear). A precise knowledge of the viscosity/shear rate relationship is important both as a practical basis for rheological characterization and for a fundamental understanding of non-Newtonian behavior. Essentially we require a flow equation relating viscosity to shear rate and such equation should meet the following requirements:

- It should give an accurate fit of experimental data over a wide range of shear rate.
- It should involve a minimum number of independent constants.
- The appropriate constants should be readily evaluated.
- The constants should have a physical significance.

The flow curves for a wide range of pseudoplastic systems can be represented by the Cross model:

$$\eta = \frac{\eta_0}{1 + |t_1 \dot{\gamma}|^m}$$  \hspace{1cm} (3.36)

Where $\eta$ is the viscosity at shear rate $\dot{\gamma}$. The parameters are:

- $\eta_0$ Zero-shear-rate viscosity (limiting viscosity at $\dot{\gamma} = 0$).
- $t_1$ Characteristic time with units of time.
- $m$ Shear rate exponent.

A simple relation is the empirical power law model written as [OSTWALD, 1924]:

$$\eta = a \dot{\gamma}^{(n-1)}$$  \hspace{1cm} (3.37)

which states that the shear stress is proportional to the $n$-th power of the shear rate. An approximate relationship between the exponent $n$ and $m$ is: $m \approx 1-n$; then the Cross model can be written as:
\[ \eta = \frac{\eta_0}{1 + [t, \dot{\gamma}]^{(l-n)}} \]  

(3.38)

The Cross model was originally derived from a consideration of particle interaction in a dispersed system, but in practice was found to give good representations for polymer solutions and melts as well.

3.3.2 Wagner’s Relationship [WAGNER, 1977]

It is well known that three independent stress functions, viscosity (\(\eta\)), the first, and second normal stress differences (\(N_1\) and \(N_2\)), are sufficient to characterize the steady shear flow of fluids. Correlations between these three stress functions are obtained by more specific constitutive assumptions, which can be tested on their usefulness by comparing theoretical predictions with experimental evidence. One of these relations between the primary normal stress difference (\(N_1\)) and the viscosity (\(\eta\)) is the Wagner relationship:

\[ N_1(\dot{\gamma}) = -\dot{\gamma}^2 \frac{\partial \eta(\dot{\gamma})}{\partial \dot{\gamma}} \]  

(3.39)

The primary normal stress difference can be obtained from viscosity data by simple differentiation with respect to shear rate. The material parameter \(m\) (Wagner parameter) is associated with the strain dependence of the memory function. If we use the Cross model to obtain the differentiation, then we can predict \(N_1\) via:

\[ N_1(\dot{\gamma}) = \frac{\eta_0 \dot{\gamma}^2 (1-n)}{m(t, \dot{\gamma})^n [1 + (t, \dot{\gamma})^{(l-n)}]^2} \]  

(3.40)

It is important to specify that the slope for the primary normal stress difference is uniquely related to that of the viscosity in the power-law region [CARREAU et al., 1997].
3.3.3 Generalised Maxwell Model

The first attempt to obtain a viscoelastic constitutive equation appears to have been that of Maxwell [BIRD et al., 1987] who in 1867 developed a theory for viscoelasticity, because he thought that gases might be viscoelastic. He proposed that fluids with both viscosity and elasticity could be described by:

$$\tau + \lambda_1 \frac{\partial}{\partial t} \tau = -\eta_0 \dot{\gamma}$$  \hspace{1cm} (3.41)

Where:

$\tau$  
Stress tensor.

$\dot{\gamma}$  
Rate-of-strain tensor.

$\lambda_1$  
Relaxation time.

$\eta_0$  
Zero-shear-rate viscosity.

The structure of macromolecules in a melt or in solution is more complex than that described by a simple Maxwell model. Indeed, we do not expect a polymeric system of a broad molecular weight distribution to be characterized in terms of a single relaxation time $\lambda_1$.

The generalization is based on the idea that polymer macromolecules are of different length and possibly form a network with junction or interactions of variable resistance. Then we can write equation 3.41 for $k$-th partial stress $\tau_k$ using constants $\lambda_k$ and $\eta_k$; then we get the total stress by summing the partial stresses.

$$\tau + \lambda_k \frac{\partial}{\partial t} \tau_k = -\eta_k \dot{\gamma}$$  \hspace{1cm} (3.42)

In the case of small-amplitude oscillatory motion it is possible to obtain expressions for the real and imaginary parts of the complex viscosity (as function of the frequency). They are given by:

$$\eta'(\omega) = \frac{G'^*}{\omega} = \sum_{k=1}^{N} \frac{\eta_k}{1 + (\lambda_k \omega)^2}$$  \hspace{1cm} (3.43)
Where the storage modulus $G' = \omega \eta''$ and the loss modulus $G'' = \omega \eta'$ are its real and imaginary parts respectively. These material functions are used for material characterization, and are related to the molecular structure [CARREAU et al., 1997]

3.3.4 Relations between Material Functions

Steady shear rheological properties and small-amplitude oscillatory properties of fluids can be related. The steady viscosity function, $\eta$, can be related to the complex viscosity, $\eta^*$, and the dynamic viscosity function, $\eta'$, while the primary normal stress coefficient, $\Psi_1$, can be related to $\eta'$ and $\eta''$. Establishing relations between material functions is very advantageous. Unfortunately most of these relations are empirical and valid only for a limited class of materials and/or in limited ranges of conditions. These relations can provide data outside the measured experimental window.

3.3.4.1 Cox-Merz Rule [COX et al., 1958]

The Cox-Merz rule is the most useful empiricism; which states that the magnitude of the complex viscosity is equal to the steady viscosity at corresponding values of frequency and shear rate:

$$\eta(\dot{\gamma})_{\omega=0} = |\eta^*(\omega)| = \sqrt{[\eta'(\omega)]^2 + [\eta''(\omega)]^2} \quad (3.49)$$

This empiricism seems to work well for homogeneous polymer solutions and melts [Carreau, 1997]. For highly elastic fluids and polymer melts, it is very difficult to measure the steady shear viscosity at high shear rates using a rotational device. In this case the shear viscosity can be estimated from the Cox-Merz relation.

3.3.4.2 Laun’s Rule [LAUN, 1986]

It is desirable to predict the normal stresses in steady shear flow from more easily measured linear viscoelastic functions. Comparison of the elastic properties $(N_1(\dot{\gamma}) / 2)$ and $G'(\omega)$ shows that they have the same values at low shear rates and frequencies for a large number of polymer solutions and melts [BIRD et al., 1987]; the shape of the curves are similar. An useful
and analogous empiricism that predicts the primary normal stress difference from more readily available linear viscoelastic data is Laun's rule:

\[ N_1(\dot{\gamma}) = \frac{2\eta^* \dot{\gamma}^2}{\omega} \left[ 1 + \left( \frac{\eta^*}{\eta'} \right)^2 \right]^{0.7} \mid_{\omega = \dot{\gamma}} \quad (3.50) \]

### 3.3.5 Master Curves for the Rheological Functions

Rheological curves have similar shapes at different temperatures and concentrations. The similarity provides the basis for an important empirical method, known as the method of reduced variables, for combining data taken at several different temperatures and concentrations into one master curve for the sample.

- In order to obtain a master curve for the viscosity function at an arbitrary reference temperature \((T_0)\), we use a shift factor given by:

\[ a_T = \frac{\eta_0(T)}{\eta_0(T_0)} \quad (3.51) \]

Where:
- \(a_T\) Shift factor.
- \(\eta_0(T_0)\) Zero-shear-rate viscosity at reference temperature.
- \(\eta_0(T)\) Zero-shear-rate viscosity at any temperature.

The zero-shear rate viscosities used in all these master curves are obtained using the Cross model.

Thus the method of reduced variables predicts that a single master curve can be obtained by plotting:

\[ \frac{\eta(\dot{\gamma}, T)}{a_T} \text{ versus } \dot{\gamma} \quad a_T \quad (3.52) \]
• In the case of concentration, the fact that the power-law slope approaches a constant values at high concentrations and molecular weights suggests that for a given polymer-solvent system in the concentrated regime data taken at different concentrations or molecular weights should lie on a single curve when we plot:

\[
\frac{\eta}{\eta_0} \quad \text{versus} \quad (\dot{\gamma} t_1) \quad (3.53)
\]

• It is also possible to obtain a single master curve for the temperature and concentration effects. We found that using a reference temperature \((T_0)\) and a reference concentration \((C_0)\), then the master curve is obtained via:

\[
\frac{\eta}{a_r a_c} \quad \text{versus} \quad (\dot{\gamma} a_r a_c t_1) \quad (3.54)
\]

Where the shift factor due to concentration effects \((a_c)\) is given by:

\[
a_c = \frac{\eta_0(T_0, C_0)}{\eta_0(T_0, C)} \quad (3.55)
\]

Where:

\(\eta_0(T_0, C_0)\) Zero-shear-rate viscosity at reference temperature and reference concentration.

\(\eta_0(T_0, C)\) Zero-shear-rate viscosity at reference temperature and any concentration.

• Master curves for other material functions are obtained similarly to shear viscosity. It is possible to obtain a master curve for the primary normal stress difference at an reference temperature \((T_0)\). A master curve is obtained by plotting:

\[
\frac{N_1(T_0)}{2T} \quad \text{versus} \quad (\dot{\gamma} a_r) \quad (3.56)
\]

• For linear viscoelastic properties the master curves can be obtained by plotting:
3.3.6 Arrhenius Dependence

Exponential functions have been used for describing the temperature dependence. An Arrhenius type of relation can be used to model the temperature shift factor \( a_T \) and is described by:

\[
a_T = A_0 \exp \left( \frac{E_a}{RT} \right)
\]  

(3.58)

Here \( E_a \) is the activation energy for flow. This parameter depends on the molecular parameters of the polymer and therefore may change considerably within each class of polymer [BIRD et al., 1987]. \( A_0 \) is known as the pre-exponential factor, \( R \) is the gas constant, and \( T \) the absolute temperature.

3.4 Results and Discussion

3.4.1 Steady-Shear Viscosity Modeling

For the steady-shear viscosity experimental results the Cross model was found to be adequate for the viscosity curves. An example of these curves is presented in figure 3.1 that corresponds to a 2.0% chitosan solution in 1.0% acetic acid at different temperatures.

Tables 3.1 to 3.3 report the parameters of the Cross model obtained for chitosan solutions at the different temperatures, chitosan concentrations, ionic strength, and acidic solutions used. The fittings were performed using Sigma Plot 5.0 software. The tables show how the zero-shear-rate viscosity \( \eta_0 \) decreases with increasing temperature and decreasing chitosan concentration. These curves are in accordance with the phenomena explained in sections 2.6.1 and 2.6.2.
Figure 3.3 Zero shear-rate viscosity ($\eta_0$) obtained with the Cross model versus temperature for chitosan in 1.0% hydrochloric acid solution at different chitosan concentrations with (0.2M NaCl) and without salt.

Figure 3.4 Characteristic time ($t_1$) obtained with the Cross model versus temperature for chitosan in 1.0% lactic acid solution at different chitosan concentrations with (0.2M NaCl) and without salt.
Table 3.1 Cross-model parameters for chitosan in 1.0% acetic acid solution at different chitosan concentrations, ionic strengths, and temperatures.

<table>
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<th>Chitosan Concentration</th>
<th>Salt</th>
<th>T (°C)</th>
<th>$\eta_0$ (Pa s)</th>
<th>$t_1$ (s)</th>
<th>$n$ (-)</th>
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Table 3.2 Cross-model parameters for chitosan in 1.0% lactic acid solution at different chitosan concentrations, ionic strengths, and temperatures.

<table>
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<th>Chitosan Concentration</th>
<th>Salt (M)</th>
<th>T (°C)</th>
<th>η (Pa s)</th>
<th>t₀ (s)</th>
<th>n (-)</th>
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Table 3.3 Cross-model parameters for chitosan in 1.0% hydrochloric acid solution at different chitosan concentrations, ionic strengths, and temperatures.

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<th>$t_1$ (s)</th>
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<td>3.8E-02</td>
<td>7.8E-04</td>
<td>2.3E-01</td>
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</table>
Figure 3.3 shows a similar plot in the case of chitosan in 1.0% hydrochloric acid solution, a strong acid. The zero-shear-rate viscosity also decreases when increasing the temperature and decreasing chitosan concentration. However when we compare the effect of salt addition we can observe that there are almost no differences with respect to chitosan concentration, phenomena also described in section 2.6.4. From these figures we can observe that the zero-shear-rate viscosity parameter is able to describe the effect of the temperature, concentration, and salt on chitosan solutions.

The characteristic time ($t_1$), another parameter of the Cross model has a similar response with respect to temperature, concentration and ionic strength effects. Figure 3.4 shows the relationship between $t_1$ and temperature for chitosan in 1.0% lactic acid solution at different chitosan concentrations, with or without salt (0.2M NaCl). The last parameter of the Cross model corresponds to the slope ($n - 1$) in a graph of log $\eta$ against log $\dot{\gamma}$: the linear region at high shear rates. The values of this parameter remains almost constant with a slope ($n - 1$) between 0.75 and 0.90. This is in agreement with the values generally obtained for polymers solutions and melts [CROSS, 1969].

3.4.1.1 Master Curves for Temperature Effect and Arrhenius Dependence

As described in section 3.3.5, it is possible calculate a shift factor due to temperature effects ($a_T$) using the zero-shear-rate viscosity parameter ($\eta_0$) obtained from Cross model. We chose 25°C as the reference temperature ($T_0$).

For all the systems studied it was possible to obtain master curves for temperature effects. An example of these curves is given in figure 3.5 for chitosan in 1.0% acetic acid solution with 0.2M NaCl. In this plot we can observe four curves that correspond to the different chitosan concentrations (2.0, 1.5, 1.0, 0.5%), each curve including the values obtained for all the temperatures (25, 30, 37, 45°C).

The effect of the addition of a salt (0.2M NaCl) can be observed also in master curves. Figure 3.6 presents the master curve for chitosan in 1.0% acetic acid solution with and without salt. As in figure 3.2, with a decrease in chitosan concentration the difference in viscosity between the solution with salt and the solution without salt is important.
On the other hand, the magnitude of the activation energy for flow ($E_a$), obtained with the Arrhenius relation described in equation 3.58 reflects the influence of the temperature on the intermolecular interaction of the chitosan macromolecules in the solvent. In general, for solutions of small concentration that shows nearly Newtonian behaviour, has activation energy for flow lower than concentrated solution containing more entanglements of macromolecules, which behaves as non-Newtonian fluid. The effect of the salt addition also is notable at low concentration, $E_a$ for systems with salt are higher that the same system without salt. The values obtained for our systems are presented in table 3.4.

Molecules have to move from one position to another through a barrier of activation energy reaching equilibrium level in accordance to the free volume theory [FRENKEL, 1946]. $E_a$ values show that the molecular structure of the solution (due to a polymer-solvent interaction and a change of density of entanglements) is function of concentration and ionic strength has important changes.

3.4.1.2 Master Curves for Concentration Effects

The zero-shear-rate viscosity parameter ($\eta_0$) and the characteristic time ($t_1$) obtained with Cross model are necessary to obtain the master curves for the concentration effect, as it was described in equation 3.53. The curves are obtained when by plotting ($\eta / \eta_0$) versus ($\dot{\gamma} t_1$). The master curves were obtained for all the systems studied. An example of these master curves is presented in figure 3.7 for chitosan in 1.0% acetic acid solution at 25°C. As it can be seen all chitosan concentration (2.0, 1.5, 1.0, and 0.5%) produce a unique master curve that represents the data.

3.4.1.3 Master Curves for Temperature and Concentration Effects

All the steady viscosity made with chitosan solutions can be summarised and regrouped in 6 master curves for which the temperature and concentration effects are included, as explained in equations 3.54 and 3.55. These master curves correspond to the different chemical systems studied:

- Chitosan in 1.0% acetic acid solution (figure 3.8).
- Chitosan in 1.0% acetic acid solution with 0.2M NaCl (figure 3.9).
- Chitosan in 1.0% lactic acid solution (figure 3.10).
- Chitosan in 1.0% lactic acid solution with 0.2M NaCl (figure 3.11).
- Chitosan in 1.0% hydrochloric acid solution (figure 3.12).
- Chitosan in 1.0% hydrochloric acid solution with 0.2M NaCl (figure 3.13).

The reference temperature and reference chitosan concentration were 25°C and 2.0% respectively.

Table 3.4 Activation energy for flow ($E_a$) in chitosan solutions.

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<th>NaCl Solution</th>
<th>Chitosan Concentration (%)</th>
<th>$E_a$ (kJ/mol)</th>
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<td>40.2</td>
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<tr>
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Figure 3.8 Master curve including temperature and concentration effects for chitosan in 1.0% acetic acid solution at different chitosan concentrations without salt.

Figure 3.9 Master curve including temperature and concentration effects for chitosan in 1.0% acetic acid solution at different chitosan concentrations with salt (0.2M NaCl).
Figure 3.12 Master curve including temperature and concentration effects for chitosan in 1.0% hydrochloric acid solution at different chitosan concentrations without salt.

Figure 3.13 Master curve including temperature and concentration effects for chitosan in 1.0% hydrochloric acid solution at different chitosan concentrations with salt (0.2M NaCl).
Figure 3.15 Primary normal stress difference versus shear rate for 1.5% chitosan in 1.0% lactic acid solution at different temperatures. Lines correspond to Wagner's relationship and symbols to experimental data.

Figure 3.16 Primary normal stress difference versus shear rate for 1.0% chitosan in 1.0% lactic acid solution at different temperatures. Lines correspond to Wagner's relationship and symbols to experimental data.
Figure 3.17 Master curves for temperature effects over the primary normal stress difference for chitosan in 1.0% lactic acid solution without salt. □ 2.0% chitosan, △ 1.5% chitosan, ○ 1.0% chitosan.

Figure 3.18 Master curves for temperature effects over the primary normal stress difference for chitosan in 1.0% lactic acid solution with salt (0.2 M NaCl). □ 2.0% chitosan, △ 1.5% chitosan, ○ 1.0% chitosan.
Figure 3.19 Storage modulus ($G'$) and loss modulus ($G''$) for a 1.5% chitosan in 1.0% hydrochloric acid solution at different temperatures. The symbols represent experimental data and the lines represent the modelisation.

Figure 3.20 Loss modulus ($G''$) for a chitosan in 1.0% hydrochloric acid solution at different chitosan concentrations, without salt and with salt (0.2M NaCl) and at 25°C. The symbols represent experimental data, and the lines represent the modelisation.
In figure 3.20 we can see the effect of the addition of salt (0.2M NaCl) in hydrochloric acid solutions (strong acid). The loss modulus ($G''$) is unchanged for solutions with salt and without salt with respect to the chitosan concentration. As explained in section 2.6.4, this phenomena is related to the suppression of electrostatic effects when chitosan is dissolved in hydrochloric acid; it is not affected by salt addition.

In figure 3.19 we can also see how well the model fits for the case of loss modulus ($G''$) while some differences are obtained for the storage modulus ($G'$). $G''$ gives information about the viscous character of the fluid and on the other hand, is related to the pure viscosity ($\eta'$), the real part of the complex viscosity ($\eta^*$). $G'$ gives information about the elastic character of the fluid. It can be seen for these chitosan solutions, viscous effects are predominant over plastic effects. The last affirmation will be confirmed later when we will present the plots of the Cox-Merz rule (section 3.4.4). In these plots (for example figure 3.23) we can observe, for all the systems studied, that the steady shear viscosity ($\eta$) coincides with the complex viscosity ($\eta^*$), and that the complex viscosity is almost equal to the pure viscosity ($\eta'$) giving negligible elastic effects represented by the elastic viscosity ($\eta''$).

Tables 3.6 to 3.8 show the generalised Maxwell model parameters for the chitosan solutions studied. In these tables we can observe that six parameters were necessary only at high chitosan concentrations (three moduli parameters and three relaxation times), because experimental data were obtained over a large range of frequency. The other systems were modeled using only four parameters (two moduli parameters and two relaxation times). It is also important to notice that for chitosan in hydrochloric acid solutions it was not possible to obtain the model parameters for 1.5 and 1.0% chitosan solutions at 45°C. An example of the behavior observed for the moduli parameters for chitosan in acetic acid solution without salt, at different chitosan concentrations (2.0, 1.5, and 1.0%) and temperatures (25, 30, 37, 45°C) is presented in figure 3.21.

In general, the moduli decrease with a decrease in chitosan concentration and increase with temperature. The same behavior was observed with the zero-shear-rate viscosity obtained with the Cross model for steady-shear tests (see figures 3.2 and 3.3). This correlation is the base of the Cox-Merz rule and will be discussed in section 3.4.4.
Furthermore, tables 3.6 to 3.8 show how large $G_k$ values are related to short $\lambda_k$ values. This means that the low-frequency behavior is dominated by the long relaxation times and the high-frequency response is controlled by the short relaxation times.

![Figure 3.21 Moduli parameters ($G_k$) for chitosan in 1.0% acetic acid solution at different chitosan concentrations and temperatures.](image)

3.4.3.1 Master Curves for Oscillatory-Shear Flows

We know that for linear viscoelastic properties, master curves can be obtained by plotting the loss modulus ($G'$) versus the shift parameter ($\alpha_T$) multiplied by the frequency (see equation 3.57). The shift factor ($\alpha_T$) is the same as used for master curves of steady-shear tests; 25°C still being the reference temperature. An example of these curves is presented in figure 3.22 which is the master curve for chitosan in 1.0% acetic acid solution with and without salt (0.2M NaCl).

The effect of salt over the master curves is similar as that observed in figure 2.21 for experimental data of loss moduli ($G''$). When the chitosan concentration decreases a possible difference is observed between the loss moduli ($G''$) data with and without salt. The presence
of salt acts similarly for the G' and the G'' data. It was possible to obtain master curves for all the chitosan solutions studied.

Table 3.6 Generalised Maxwell model parameters for chitosan in 1.0% acetic acid solution at different chitosan concentrations, ionic strengths, and temperatures.

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Table 3.7 Generalised Maxwell model parameters for chitosan in 1.0\% lactic acid solution at different chitosan concentrations, ionic strengths, and temperatures.

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3.4.4 Comparison between Steady and Oscillatory Data

Normal stress functions arise from the viscoelastic nature of materials. It is reasonable to expect that linear viscoelastic material functions determined from oscillatory experiments be related to steady shear behavior. Cox and Merz [1958] observed that the complex viscosity is

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Figure 3.22 Master curve for temperature effects over the loss modulus ($G''$) in the case of chitosan in 1.0% acetic acid solution with (0.2M NaCl) and without salt at different chitosan concentrations.

Figure 3.23 Comparison of the Cox-Merz rule for a 2.0% chitosan in 1.0% lactic acid solutions at different temperatures.
Figure 3.24 Comparison of the elastic properties $(N_1/2)$ and $G'(\omega)$ for 2.0% chitosan in 1.0% lactic acid solutions at different temperatures.

Figure 3.25 Comparison of Laun's rule and the primary normal stress difference for 1.5% chitosan in hydrochloric acid solutions. The open symbols are Laun's rule data; the closed symbols are the experimental data. The symbols correspond to different temperatures: ■ 25°C, ◆ 30°C, ▲ △ 37°C, ○ 45°C.
These results are consistent independently of chitosan concentration and temperature. At the conditions shown in the table the visualization of the differences is clearer due to the effects explained in the section 3.4.1 of this thesis. We make a comparison using the parameter $\eta_0$ because the higher or lower values of $\eta_0$ always coincide with the higher or lower values of the rheological properties derived from experimental tests (steady-shear viscosities, dynamical moduli and primary normal stress differences). We can observe that lower values of $\eta_0$ are obtained for:

- Systems with NaCl, and
- Solutions where the chitosan is dissolved in a strong acid (HCl solution)

Thus we can say that the use of a strong acid (total dissociation of the HCl molecule in the aqueous medium) and the use of a salt are ways of increasing the ionic strength of our solutions. We know that due to this increase the protonated amine group is totally neutralized and the electrostatic repulsion forces tends to disappear. Furthermore we can say that in our chitosan solutions there are conformational transitions between the rod conformation (extended polymer chains that produce higher viscosities in the fluids) and the random coil conformation (compacted and less rigid polymer chains that produce lower viscosities in the medium).

These conformational changes are observed independently of the temperature and chitosan concentration, and detected simply by the viscosity changes of the solutions.

There are other important changes in our solutions that are independent of ionic strength. These changes are due to the shear rate applied during the rheological tests. We can observe in figures 2.9, 2.16 or 3.1 different examples of the steady-shear tests. There are two regions:

- At low shear rates we observe a Newtonian behavior (viscosity independently of the shear rate).
- At high shear rates we observe a non-Newtonian behavior that can be modeled by a power law.
We assume that the transition between the Newtonian region and the power law region is due to a conformational change, and this conformational change goes in the following direction: at low shear rates we observe a Newtonian behavior with high viscosities which can be explained by the model of the rod chains that preserve the structure with the low forces that are applied.

However at high shear rates we observe a non-Newtonian behavior with lower viscosities. This behavior can be explained for the conformational transition induced by the increases in shear rates (forces supported by the fluid); the rigid rod chains will change to a random coil conformation where the chains which become more compacted. This effect will produce a viscosity decrease.

In summary and in accordance with the literature review for the conformational studies of chitosan in solutions and with the results obtained in this study we can say that chitosan solutions change between a rod-like and a random coil conformation. These changes are functions of the degree of acetylation of the chitosan and the ionic strength of the solutions. We can also say that these changes are also function of the forces applied to the systems (shear rates or frequencies used in the rheological testing).

This concludes the presentation and discussion of the rheological study of chitosan solutions. A wide range of experimental conditions and models were presented in the last two chapters. As shown, the data and the models cover a large range of experimental conditions (shear rates, frequencies, temperature, etc.).

For the experimental conditions studied, chitosan solutions behaved like non-Newtonian shear-thinning fluids with normal stresses. This can be related to strong intermolecular hydrogen bonding even at low chitosan concentrations, because chitosan macromolecules have a tendency to form entanglements. Intermolecular interactions control the rheological response of chitosan in solutions as indicated by the variation of viscosity and shear moduli with changes in acidic medium and ionic strength at a given concentration and temperature.

In the following chapter we will present experimental results obtained from a rheological study of a polyelectrolyte complex system: chitosan-xanthan hydrogels dispersed in different aqueous medium. The viscoelastic properties and yield stresses of these materials will be
presented. The effect of parameters like hydrogel concentration or dispersion medium will be discussed. For the sake of brevity this chapter will only present some examples for the models. All the results for the models and empirical relationships are included in an annex document (CD disc: AgustinMartinez_RheoChitoxan_1997-2001).
4. VISCOELASTIC PROPERTIES OF CHITOSAN/XANTHAN HYDROGELS

4.1 Summary

Polyelectrolyte complex formation is one way to prepare sensitive hydrogels. In this work a gel was formed by complexation from two natural polyelectrolytes, chitosan and xanthan. Chitosan is a weak basic polysaccharide with amino groups, while xanthan gum is an acidic polysaccharide with carboxyl groups. When solutions of these two polysaccharides are mixed, aggregation of the two polyelectrolytes immediately occurs because of strong attraction between the two oppositely charged polyelectrolytes.

Changes in the rheological properties of hydrogels have been studied. These changes are function of experimental conditions such as:

- Hydrogel concentration (5 to 10% w/w).
- Type of chitosan used in the hydrogel formation (two chitosan samples with different molecular weight and degree of acetylation)
- Range of particle size of the dry hydrogel (125-75 μm and 75-55 μm)
- Chemical media used for the hydrogel dispersion (distilled water, buffer solution at pH = 7.0, and 0.05M NaCl solution)
- 'Waiting time', that is the time that elapses between the moment when the hydrogel is dispersed in the chemical media and the moment when the rheological test is performed (up to 390 minutes).

The viscoelastic properties of this polysaccharide system were characterized by oscillatory shear measurements under small-deformation conditions. The shape of mechanical spectra (storage modulus variations as a function of frequency) offers a good means to describe the overall properties of the systems. Hydrogels show a linear dependence of G' over all the frequency range. These mechanical spectra show a decrease in G' with decreasing hydrogel concentrations. They also show a decrease in G' at low “waiting time”, but G' reaches an almost constant value at high “waiting time”.

The rheological experiments also include the yield stress determination using a steady-shear method that measures the hydrogel deformation at different shear stresses.

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a) A gel consists of two or more components, one of which is a liquid, present in substantial quantity.

b) A gel is a soft, solid, or solid-like material.

c) Solid-like gels are characterised by the absence of an equilibrium modulus, by a storage modulus (\(G'\)) which exhibits a pronounced plateau extending to times at least of the order of seconds, and a loss modulus (\(G''\)) which is considerably smaller than the shear modulus in the plateau region.

4.2.1.2 Classification of Gels

Flory [1974] in accordance with his early work proposed a classification of gels based on structural criteria:

I. Well-ordered lamellar structures. For example, we have soap gels and phospholipids.

II. Completely disordered covalent polymeric networks: Gels of this type have complete disorder. Examples are: vulcanised rubbers, the protein elastin, etc.

III. Polymer networks formed through physical aggregation; predominantly disordered, but with regions of local order. Those gels are reversible; they can be dissolved and reformed by cycling the temperature or the solvent.

IV. Particulate, disordered structures. The fourth category of gels generally consists of particles of large geometric anisotropy; includes flocculant precipitates and aggregation of proteins. An example is a suspension of rods that exhibit the properties of a gel in a certain concentration range.

The principal difference between Flory II and Flory III gel types is the physical character of the interconnections responsible for the formation of the network in type Flory III, in contrast to the covalently linked networks in type Flory II. Flory III and IV gels consist of chains aggregates physically rather than chemically crosslinked, and where the crosslinks are of small but finite energy, and/or finite lifetime [ROSS-MURPHY, 1998a].

Silberberg [1989] and Djabourov [1991] made a classification of the gels based on their phenomenological behavior: there are permanent gels and reversible gels.

1- Permanent gels are most commonly covalently linked. Such gels may appear to be homogeneous at the supermacromolecular level but are inevitably disparate at some macroscopic scale.
2- A reversible gel based upon dissociable crosslinks is characterised by the finite size of the network that is chemically linked into a unit at any instant. In a reversible gel, some of the network connections will always open, close, and interchange.

Discrimination between various types of gels and networks can be made by rheology. Djabourov [1991] presents features which characterise the sol-gel transition and which are common to a large number of gelling systems: these are the mechanical properties of the medium. Two parameters are relevant: the viscosity ($\eta$) below the gel point and the relaxation shear modulus ($E$) above the gel point.

\[ \eta = \lim_{\omega \to 0} \frac{G''}{\omega} \]  
\[ E = \lim_{\omega \to 0} G'(\omega) \]  

where $G'$ and $G''$ are the storage modulus and the loss modulus measured in the dynamical regime, $\omega$ is the angular frequency related to the frequency $f$ ($\omega = 2\pi f$).

Typical spectra of $G'$ and $G''$ are illustrated in figure 4.1. For purely entangled systems, such as polymer melts, as the frequency increases there is a crossover in $G'$ and $G''$. At very low frequencies (in the terminal zone) they flow as high viscosity liquids. By contrast true gels should not show such an effect, and for a rubbery gel, $G'$ and $G''$ are parallel, possess an equilibrium modulus, and are largely frequency insensitive [ROSS-MURPHY, 1995].

### 4.2.1.3 Physical Gels

Physical gels consist of chains which are physically crosslinked into networks, the crosslinks themselves being of small but finite energy, and/or of finite lifetime. The presence of non-covalent crosslinks complicates any physical description of the network properties, because their number and position can fluctuate with time and temperature. In many cases the nature of the crosslinks is not known unambiguously, but can include Coulombic, dipole-dipole, van der Waals, charge transfer, hydrophobic and hydrogen bonding interactions.
Figure 4.1 Dynamic mechanical spectra expected for $G'$ and $G''$ for (a) an entanglement network system; (b) a covalently crosslinked network. [ROSS-MURPHY, 1992]

For biopolymer gels in particular, non-covalent crosslinks are formed by one or more of the mechanisms listed above, almost invariably combined with more specific and complex organisational arrangements involving extended semi-crystalline "junction zones".
For biopolymer gels, in particular, non-covalent crosslinks are formed by one or more of the mechanisms listed above, almost invariably combined with more specific and complex organisational arrangements involving extended semi-crystalline "junctions zones".

A further discrimination can be made between strong and weak physical gels [CLARK et al., 1987]. Both respond as solids at small deformations, but:
- Strong gels are also solids at large deformation.
- Weak gels are structured fluids, so they flow almost as liquids at large deformation.

4.2.2 Rheological Methods for Gels

Mechanical and rheological testing methods have been used to characterise gels for a number of reasons:
- Since natural polymers have characteristics that vary with the origin of the raw material and the method of extraction, it is essential to have a reliable test for evaluating the gel strength.
- The measurements can be used to complement the information on the internal structure of a gel obtained from the application of structural characterisation techniques.

The rheology incorporates small strain tests and large strain fracture tests. Small strain tests have been used primarily to follow sol to gel transformations and structural properties of the final gel, whereas large strain tests describe the breakdown of the gel structure.

4.2.3 Gelation Experiments

4.2.3.1 Formation and Time Evolution of Gels

In biopolymer gels and specifically in polysaccharide gels crosslinking is induced either by temperature changes and/or by the introduction of specific counterions or pH variations. In most cases, lowering the temperature of a solution drives the polymer to a more ordered shape through a disorder-to-order conformational transition, and it is the formation and/or association of such ordered section of the polymer chains which is the origin of the crosslinking process. Polysaccharide gels from agarose, carrageenans, amylose, and gellan
These models must take in consideration different aspects observed in the experimental behavior of $G'$:

a) The time evolution of $G'$ tends asymptotically towards a plateau value, with a continually decreasing derivative $dG'/dt$, or it can be a sigmoidal shape.

b) The evolution process is either unique or, two consecutive phases can be observed.

c) The shape of $G'(t)$ may remain unchanged as the polymer concentration or other parameter are changed, so that it is possible to build up a master curve for a generalised description of the gelation process.

### 4.2.3.3 Gel Point Determination

Several different methods and rheological criteria have been proposed in the literature for the gel point determination. For physical gelation processes, the experiments based on small deformation oscillatory flow measurement at constant frequency present notable advantages because the variations of the viscoelastic properties may be monitored continuously without any interruption from the beginning of the test to the final gel state.

For chemical gels, a gel point criterion is the equality condition $G' = G''$. This is the Tung and Dynes criterion [TUNG et al., 1982]. In this criterion the sol/gel transition is identified with the incipient prevalence of the elastic component over the viscous one, and the crossover of the $G'(t)$ and $G''(t)$ curves marks the time of gelation. This criterion has been also applied for physical gels; for example for amylose [DOUBLIER et al., 1989], and pectin-calcium systems [DURAND et al., 1990].

Winter and Chambon [CHAMBON et al., 1985; CHAMBON et al., 1987; WINTER et al., 1986] developed a more general criterion. Starting from the analysis of the evolution of linear viscoelastic properties during chemical crosslinking, they proposed:

$$
t(t) = S \int_{-\infty}^{t} (t - t')^{-n} \dot{\gamma}(t') dt'
$$

(4.3)

The equation describe the stress in a critical gel that is deformed at a rate $\dot{\gamma}(t')$, with $-\infty < t' < t$ and $n$ lying between 0 and 1. The gel strength ($S$) and the relaxation exponent ($n$) are
network characteristic properties. As a consequence, we find that, at the gel point, the
dynamic modulus is parallel and proportional to frequency over the entire frequency range,
according to the power law relationship:

\[ G'(\omega) \sim G''(\omega) \sim \omega^n \] (4.4)

Scanlan and Winter [1991] observed that the relaxation exponent \( n \) could vary to a lesser
extent \( 0.19 \leq n < 0.92 \) depending on stoichiometry and decreases with polymer molecular
weight and concentration.

4.2.4 Experiments in Formed Gels

4.2.4.1 Dynamic Properties

When the gel has formed, the frequency of oscillation may be altered, and the shear and loss
modulus plotted as functions of frequency form the "mechanical spectrum". Strong and weak
gels can be unambiguously classified as such on the basis of their mechanical spectrum. As
far as the curing process is concerned, many biopolymers systems show increasing \( G' \) as
function of time, for days or weeks. This may be related to a number of factors including loss
of solvent. However, \( G' \) will eventually tend to decrease with time, because microbiological
attack could cause the breakdown of the chain backbone [CLARK et al., 1987].

The typical strong gel spectrum, over the frequency range say \( 10^{-2} \) to \( 10^{2} \) rad/s consists of
two nearly horizontal straight lines. \( G' \) is typically 1-2 orders of magnitude greater than \( G'' \),
and both may show a slight increase at higher frequencies (see figure 4.1). If the frequency
range is extended over \( 10^{2} \) rad/s, the effect of both dynamic entanglements and more local
intrachain vibrational modes will be seen. Sometimes, \( G'' \) will show a slight minimum in the
experimental frequency range: an example is gelatin [RICHARDSON et al., 1981a]. Another
very common example of this behavior involves calcium alginate gels at 1.4% [SEGEREN et
al., 1974].

Quite often, the dynamic analysis of strong gels is restricted to the elastic component \( G' \). This
is not only justified by the relative values of \( G' \) and \( G'' \) mentioned above but becomes an
obligatory choice when the ratio \( G''/G' \) is lower than 0.01. When this is the case, the value of
G", which depends on the instrumental resolution of the phase lag between sinusoidal stress and deformation, is no longer reliable. Strong gel behavior is not always associated with high values of the storage modulus, for example the case of amylose gels of low concentration (1.03% and 1.33%) reported by Doublier et al. [1989].

Other polysaccharides show mechanical spectra similar but not identical to those for strong gels: the profiles of both moduli show a slight frequency dependence with no sign of a Newtonian plateau in the low frequency range with G' exceeding G" at all frequencies. For example the guar gum-borate system [CARNALI, 1992], the extracellular polysaccharides from Bacillus polymyxa [MADDEN et al., 1986], and the case of xanthan solutions and gels [NORTON et al., 1984]. Such materials are weak gels and their behavior is intermediate in properties between the two extremes settled by entanglement networks and true gels; the origin of these properties can be traced to the conformational behavior of the polymer.

4.2.4.2 Concentration Effect

Clark et al. [1983, 1985] have made measurements of the shear modulus of agarose gels at various concentrations and have shown that the critical concentration C₀ is very low (0.2%). The power law dependence of the modulus on concentration tends to the limiting form G' \( \propto C^2 \) at higher concentrations. But, we have that G' \( \propto C^n \) at lower concentrations with n very much greater than 2.

Ellis et al. [1985] in the case of amylose (concentrations from 1.5 to 7% and DP = 3000), found that on average G' varies as C⁷. Clark et al. [1989] using monodisperse amylose samples of DP = 1100, 660 and 300 obtained values for n of 5.9, 4.7, and 4.4 respectively, leading to an increase of n with DP.

4.2.4.3 Temperature Effect

The complexity of the mechanisms of formation and the structural features of physical gels as well as the nature of the crosslinks (which result from equilibrium association processes between chain segments and involve extended junction zones) may be judged as being sufficient elements to understand why the temperature dependence of the viscoelastic
properties appears to be more complex for physical gels than for chemically crosslinked networks.

For an ideal rubbery network, the shear modulus is linearly increasing with increasing temperature, even though the range of validity of the linear dependence is rather narrow for many real systems. For thermoreversible gels, the modulus generally decreases with temperature and its variation becomes more pronounced as the melting condition is approached. Eldridge et al. [1954] treated the melting temperature $T_m$ of thermoreversible gel as the melting point of a partially crystalline polymer, and related it to concentration by the following relationship:

$$\ln C = \frac{\Delta H_m}{RT_m} + c$$  \hspace{1cm} (4.5)

Where $c$ is a constant and $-\Delta H_m$ is the heat absorbed on forming a mole of junction points that stabilise the network structure of the gel. In a previous section (4.2.4.2) we showed how the concentration is related to the modulus, thus we can relate the melting temperature with the concentration and the modulus. An example of the application of this combined approach concerns the analysis of rheological properties of pectin gels by Watase et al. [1993].

4.2.4.4 Viscoelastic Behavior in Gels – Transient Properties

Gels show viscoelastic behavior and can be characterised by static experiments such as the creep recovery experiments, where a constant stress, $\tau_0$, is applied and the strain, $\gamma(t)$, is followed as a function of time. The results are expressed in terms of the creep compliance $J(t)$ defined as.

$$J(t) = \frac{\gamma(t)}{\tau_0}$$  \hspace{1cm} (4.6)

An alternative static experiment is the stress relaxation experiment where a constant strain, $\gamma_0$, is applied and the stress, $\tau(t)$, required to maintain this strain is followed as a function of time. The results are expressed in terms of the stress relaxation $G(t)$ given by:
\[ G(t) = \frac{\tau(t)}{\gamma_0} \]  \hspace{1cm} (4.7)

Gels systems are expected to follow viscoelastic solid behavior. After application of the stress in a creep experiment, the strain will eventually reach a constant value and on removal of the stress, the strain will finally return to zero. In the case of viscoelastic solutions however a permanent deformation is expected after removal of the stress. In a stress relaxation experiment \( G(t) \) will decay to zero for a viscoelastic solution whereas for a gel it will reach a constant nonzero value. The results from stress relaxation tests are usually expressed in terms of relaxation modulus, \( G \) (dynamic shear modulus).

Higgs and Ross-Murphy [1990] recommended keeping in mind the following points when performing experiments:

1- Gel formation time-scale: Measurements of gel modulus show that it continues to increase after the initial setting of the gel. It is essential to wait sufficiently long time before beginning the experiment so that any additional change during the duration of the experiment is negligible.

2- Temperature and cooling procedure. The mechanical properties of gels change rapidly with temperature; and there are differences between gel samples cooled directly to a low temperature, and those cooled in two stages, even after a considerable waiting period at the final temperature.

3- Applied stress levels. Gel modulus vary rapidly with concentration, in particular at high concentrations an approximate \( C^2 \) dependence is seen; at lower concentration the dependence is more pronounced.

4.3 Rheology of Polysaccharide Gels

Polysaccharide gels are encountered in many biological systems, including those from marine algal, cultivated plants, animal and microbial sources. In these cases gel formation is generally associated with the transformation of essentially disordered biopolymers to a partly ordered state.
4.3.1 Plant Polysaccharides

4.3.1.1- Starch

Starch consists of two different polysaccharides, one, predominantly linear, being amylose and the other branched but structurally analogous, amyllopectin. The proportion of each polymer, depends upon the source (potato, rice, etc.). Starch behavior is related to the limited compatibility and mutual gelation of the two polymeric components. [MILES et al., 1985]. The physical characteristics of starch gels depend on the rheology of the amylose matrix, the rigidity of the amyllopectin granules, and the volume fraction and shape of the granules. All these factors depend on the source of the starch, on processing conditions, and product formulation [OAKENFULL, 1987].

Ellis et al. [1985] have measured shear modulus of amylose gels and have related them to amylose concentration. A critical concentration of approximately 1.0% was indicated as well as a \( C^7 \) power law dependence of modulus on concentration for the range 1.5% to 7.0%. They also reported on the high elasticity of amylose gels, and upon the tendency for the shear modulus to fall slightly with temperature in the range up to 100°C. Amylose gels have a high melting point (in excess of 100°C) and hence show pronounced hysteresis.

4.3.1.2- Pectin

Pectins, found in fruits (apples, lemons) and other fibrous sources (sugar beet), as the intracellular cement within the plant cell wall, consist predominantly of sequences of galacturonic acid, with occasional interruption by rhamnose residues. Some of the carboxyl groups are methyl-esterified and the distribution depends upon the plant source and age. Pectins with low degree of esterification (low proportion of methoxyl groups) gel with divalent cations. The more esterified materials gel under conditions of low and the junctions zones are thermoreversibles; a very important factor is the key role of hydrophobic interactions in stabilising their junction zones [ROSS-MURPHY et al., 1998b].

Watson [1966] has studied the viscoelastic and rupture behavior of high methoxyl pectin sugar gels under three different types of loading: impact, compression, and static. The results
from creep and compression studies indicated the presence of plastic flow at low stresses. This non-recoverable strain was far greater than could be explained by the viscous flow part of the creep curve. At high stresses the response of the gel became quite elastic although very dependent upon the rate of strain.

4.3.1.3- Galactomannans

These polysaccharides (guar gum and locust bean gum) are derived from the endosperms of plant seeds, where they function as reserve during germination. They are used in the food industry as thickening agents and, in combination with other polysaccharides such as agarose and xanthan, as gelling agents.

Dea and Morrison [1975] have extensively reviewed the properties of the galactomannans, including their propensity for interaction with other kinds of polysaccharide. Since they are of quite high molecular weight and not so flexible, they have high viscosity for a given molecular weight and are therefore efficient thickeners [ROBINSON et al., 1981].

4.3.2- Microbial Polysaccharides

The microbial polysaccharides are derived primarily from bacteria where they are present outside the cell as either a discrete capsule of a diffuse slime that is secreted into the medium. The two major members of this group are gellan, an anionic polysaccharide produced by *Auromonas elodea* and xanthan from *Xanthomonas* strains.

4.3.2.1- Gellan

Gellan has a complex tetrasaccharide repeat unit, and gels in the presence of multivalent cations, via a double helical intermediate, similar to the mechanism for the carrageenans. The mechanical properties are sensitive to the degree of acylation of the chain [ROSS-MURPHY et al., 1998b].
copolymers of 1,3-linked β-D-galactose (group A) and 1,4-linked 3,6-anhydro-α-D-galactose (group B). They can be written generally as (AB)_n.

The gelation process involves the formation of double helical structures. Because of the ionic nature of the polymer, gelation is strongly influenced by the presence of electrolytes, particularly potassium ions [OAKENFULL, 1987]. Under suitable condition of ionic strength, temperature, and polymer concentration all metal alkali ions will induce gelation of carrageenans.

Morris et al. [1980, 1981, and 1983], and Elliot et al. [1975] have both made shear modulus measurements on carrageenan gels. They considered the effect of concentration, cations, and temperature on the modulus. For 𝜈-carrageenan the dependence of the cation type on elasticity modulus follows Cs⁺ > Rb⁺ > K⁺ >> Na⁺ > Li⁺ (with Na⁺ and Li⁺ under forcing conditions). This suggests that the ions, by acting as solvent structure-builder structure breakers, are influencing gelation through their effects of hydrogen bonds that stabilise the helices.

4.3.3.2- Agar

Polymers of the agar family, like the carrageenan are thermoreversible gels. They have an alternating backbone consisting of 3-linked β-D-galactopyranose and 4-linked 3,6-anhydro α-L-galactopyranose. It forms firm, slightly turbid gels on heating >0.2% aqueous dispersion of the polymer above 90°C and then cooling to about 30°C. The gelation proceeds rather similarly to that of gelatin, a triple helix is involved [ROSS-MURPHY et al., 1993].

Arakawa [1961], and Watase et al. [1967 and 1968] have studied the stress relaxation response of agar. In these investigations a sample was compressed and the change in stress with time was measured. The amount of the imposed strain was 5%, although linearity was demonstrated between the stress measured two minutes after the instantaneous compression and strains up to 15%. Measurements were made using a variety of concentrations, temperatures, and pH's.
Clark et al. [1983 and 1985] have made measurements of the shear modulus of agar gels at various concentrations. They showed that the critical concentration ($C_0$) is very low ($\approx 0.2\%$), and that the power law dependence of the modulus on concentration tends to $C^2$ at $C/C_0 > 10$, but is proportional to $C^n$ at lower concentrations (with $n \geq 2$).

4.3.3.3- Alginates

The alginates are copolymers of L-guluronic and D-mannuronic acid. They are the major structural polysaccharides of brown algae of the Phaeophyceae family. They form gels when divalent ions are slowly introduced into solutions of a monovalent salt of alginic acid. They are not thermoreversible. Gels formed from alginates containing a high concentration of guluronic acid have been described as rigid and brittle, and those formed from low guluronic acid alginates are elastic and flexible [MITCHELL, 1976a].

Mitchell and Blanshard [1974 and 1976b] made measurements of the creep compliance for one hour after application of stress using a parallel plate viscoelastomer. A linear viscoelastic behavior was observed and a linear model was able to fit the creep data obtained in the linear region with a Maxwell element in series with one or more Voigt elements. The effects of varying pH, calcium ion and alginate concentration were examined in the gels. The compliance of the gels exhibited a pronounced dependence on pH being larger at high pH than at lower pH's.

Segeren et al. [1974] reported results of dynamic viscoelasticity studies of alginate gels. In their work the modulus of gels tested was found to be independent of frequency in the range $10^{-1}$ to $10^1$ Hz, and the ratio $G''/G'$ was found to be very small. $G'$ was found to increase linearly with temperature in a large range ($20^\circ$C to $50^\circ$C) in agreement with the elasticity theory of rubber. However the departure from linearity between stress and strain was observed at values of strain of about 0.13. The gels contain crosslinks of varying strength depending on the number of calcium ions involved in the crosslink. The alginate gel networks maybe a more complex structure than what is predicted by the rubber elasticity theory.
4.3.3.4 Chitosan

More physical, chemical and rheological properties of chitosan can be consulted in the sections 2.2 and 2.3 of this work.

- Formation of Chitosan Gels.

Hydrogels are usually formed by the covalent cross-linking of linear hydrophilic polymers to form a network of material capable of absorbing water, yet still remaining insoluble. Heterogeneous polymer mixtures may also be used to form hydrogels without the need for covalent cross-linking [Noble et al., 1999].

As was reviewed in chapter 2, chitin is a biopolymer and is the second most abundant renewable organic resource on earth. Chitin can be deacetylated to produce chitosan that has a polymeric cationic character. [HAN, 1996].

Chitosan hydrogels are usually covalently crosslinked. Crosslinked agents such as glutaraldehyde, glyoxal have been employed in the fabrication of chitosan hydrogels [Nakatsuka et al., 1992; DE Angelis et al., 1998]. In addition semi-interpenetrating polymer network hydrogels and co-network of two crosslinked polymers have been produced [Noble et al., 1999]. Such hydrogel materials have limited biocompatibility and biodegradability.

Non-covalent crosslinking has been used to prepare chitosan-based gels. For example by the O- and N- acetylation of chitosan with acetic anhydride. However the gels are rigidly solidified or fragile gels with high and low molecular weight chitosan respectively [Kristl et al., 1993]. Possible attachment of C10-alkyl glycosides to chitosan; they form gels in acid media at elevated temperature but are solutions at lower temperatures [HOLME et al., 1991]. Palmitoyl glycol chitosan non-covalent gel can be prepared by attachment of palmitoyl groups to the water-soluble derivative of chitosan: glycol chitosan [Noble et al., 1999].

Non-covalent cross-linked chitosan gel mixtures can be prepared by the use of polyelectrolyte complexes of chitosan and polymers such as carrageenan or xanthan. When two oppositely charged polyelectrolytes are mixed in an aqueous solution, a
effect of the water on polymer network can be correlated with the relaxation of hydrogel network.

There are also rheological works with semi-interpenetrating networks; Khalid et al. [1999] reported swelling properties and rheological characterisation of a semi-interpenetrating chitosan-polyethylene oxide network. They showed how the network displayed a high capacity to swell, adjustable by pH. The rheological properties showed that the semi-interpenetrating network had elastic properties that are enlarged by the presence of the polyethylene oxide physical network.

Studies of the electrorheological characteristics of chitosan suspensions in silicone oil were performed by varying electric fields, volume fractions, shear rate, and temperature [CHOI et al., 1999]. The systems had a Bingham's plastic fluid behavior.

There are also a few works on the rheology of chitosan gel systems used in the pharmaceutical field. The most interesting example is presented by Bodek [2000]. In this work it is presented a study of the rheological properties of microcrystalline chitosan hydrogel prepared by adding a methylcellulose hydrogel to a water dispersion of the chitosan. The results of the rheological tests showed a system that can be pseudoplastic or plastic depending on the kind and content of the pharmaceutical substances and on the interactions between the polymer and these pharmaceutical substances.

Another example is presented by Ruel-Gariépy et al. [2000] for the case of chitosan/glycerophosphate thermosensitive solution, which gels at 37°C. They evaluated the in vitro release profiles of different pharmaceutical model compounds; this study showed the effects of parameters like temperature, chitosan deacetylation degree, molecular weight and substances present.

This review showed that there are only a small number of works in the field of rheology of chitosan gel systems. In this chapter a study on the rheological behavior of chitosan/xanthan gels using two different chitosan for the gel formation is presented. We are presenting the effect of the particle size of the gel used in formation of the dispersion, the chemical media used in the dispersion, and the hydrogel concentration on the rheology. The properties are evaluated in dynamic regime. For selected systems, yield stresses were performed.
4.4 Experimentation

4.4.1 Materials

The chitosan was made from chitin obtained from shrimp’s shells. It was produced by Kemestrie Inc., Sherbrooke (Quebec). Two chitosan samples with degree of acetylation (DA) of 28% and 25% and weight average molecular weight ($M_w$) of $8.2 \times 10^5$ g/mol and $1.08 \times 10^6$ g/mol respectively were used.

The xanthan was supplied by Kelko Inc. Xanthan gum is a high molecular weight (approximately $1 \times 10^6$ g/mol) polysaccharide produced by a pure culture fermentation of a carbohydrate with X. campestris, then purified by recovery with isopropyl alcohol, dried, and milled. It contains D-glucose and D-mannose as the dominant hexose units, along with D-glucuronic acid, and is prepared as the sodium, potassium, or calcium salt. Xanthan is completely soluble in hot or cold water. Its enzymatic synthesis by fermentation under controlled conditions provides a structurally uniform polymer with a narrow molecular weight distribution. This characteristic may contribute to the extraordinary solution stability and unusual solution behavior of xanthan [PETTITT, 1982].

For the formation of the hydrogel dispersions we used three different chemical media: distilled water, a buffer solution (0.05M potassium phosphate monobasic – sodium hydroxide buffer with pH 7.00 at 25°C by Fisher Scientific), and 0.05M NaCl aqueous solution (analytical grade sodium chloride by Sigma).

Analytical grade hydrochloric acid (BDH) and sodium hydroxide (Anachemia) were used for chitosan solubilization and neutralization.

4.4.2 Rheometry

We measured the rheology of gels using two geometries: parallel plate and cone-and-plate. The cone-and-plate rheometer description was made in section 2.4.2.2.
$\Omega$ Angular velocity of the plate.

$T$ Torque on the plate.

![Figure 4.2 Parallel plates rheometer.](image)

4.4.3 Methods

4.4.3.1 Preparation of Chitosan-Xanthan Hydrogels

- Chitosan Solutions Preparation.
  Chitosan samples were used to prepare solutions as follows: 6.5 g of chitosan were dissolved in 300 ml of 0.1N HCl. The solution was then neutralized with 0.2N NaOH, and distilled water was added for a total volume of 1000 ml at pH = 5.6. To obtain a good solution, the chitosan powder was dispersed by agitation with a magnetic stirrer until total solubilisation. The chitosan solutions were kept at 4°C until the gel formation was performed.

- Xanthan Solutions Preparation.
  A xanthan solution was prepared by adding 6.5 g of xanthan to 1000 ml of distilled water and dispersion was done with a magnetic stirrer until total solubilisation.

- Hydrogel Formation.
  Mixing 1000 ml of the chitosan solution with 1000 ml of the xanthan solution formed the hydrogel. Agitation was conducted for 10 min at room temperature; the mixture was let stand for 4 hours and then separated by filtration. The hydrogel was washed repeatedly with water to remove the free chitosan and xanthan. The washed hydrogel was lyophilized in a freeze dry system (LABCONCO) and kept in desiccator until rheological samples preparation.
• Rheological Samples Preparation.

The study was made with hydrogel of different particle size. Two different fractions were used: a first fraction with particle diameters between 125 and 75 µm, and another fraction between 75-55 µm. In order to obtain these fractions the dried hydrogel was grounded using a Pulverisette 14 by Fritsch; and sifted using 3 different USA standard testing sieves by Fisher Scientific: No. 120 (125µm), No. 200 (75µm), and No. 270 (55 µm).

The dried hydrogel was weighted in order to prepare dispersions of the hydrogel in different chemical solvent media. We used three different media: distilled water, buffer solutions (pH = 7.0) and 0.05M NaCl solutions. We prepared dispersions at different hydrogel concentrations (from 5 to 10%, as function of the hydrogel used). As soon as hydrogel are weighted it is dispersed in the media until a homogeneous dispersion of the gel in the media is obtained. For better dispersion of the hydrogel, it is necessary to perform manual agitation. For this reason we prepared only small quantities of dispersion in each batch: between 8 and 15 g.

4.4.3.2 Rheological Tests

The rheological tests were performed using two rheometers: A Carri-Med Weissenberg rheogoniometer R20 for the measure of the dynamic properties of the hydrogels: frequency sweep and time sweep tests. A Bohlin CVO HR 120 rheometer was used for the measurements of the yield stress.

• The Weissenberg rheogoniometer R20 is a strain-imposed rheometer with interchangeable test geometries. It can be used with cone-plate and plate-plate geometries. It measures shear and normal forces over a wide range of both steady and dynamic shear.

Flow measurements were carried out over a range of hydrogel concentrations (5 to 10%) at a temperature of 25°C. In order to preserve the moisture of the hydrogels, a special setup was adapted that allows circulation of water saturated air. The temperature control was performed using an extended temperature module (Carri-Med) that operates by directly heating the moving and fixed plates, and controlling the temperature with the aid of a coil for the circulation of cold water.
For the determination of the oscillatory-shear rheology we used parallel plate geometry. The gel was placed in the gap between the parallel plates of the Weissenberg. The technical specifications for the selected geometry were:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plates Diameter</td>
<td>40 mm</td>
</tr>
<tr>
<td>Plate Thickness</td>
<td>6 mm</td>
</tr>
<tr>
<td>Gap</td>
<td>1500 μm</td>
</tr>
<tr>
<td>Strain</td>
<td>3.0%</td>
</tr>
<tr>
<td>Torsion Constant</td>
<td>9.810 μN m / μm</td>
</tr>
</tbody>
</table>

Logarithmic frequency sweep measurements were carried out over frequencies ranging from 0.1 up to 65 s\(^{-1}\) by taking 15 experimental points. Preliminary strain sweeps test confirmed that we are in the linear viscoelastic regime. Time sweep measurements were performed for 1000 sec at a constant angular frequency of 1 and 10 Hz with a sequence of one experimental point each 60 seconds.

- The characteristics of the Bohlin CVO HR 120 rheometer were described in section 2.4.3.4. For the determination of the yield stress we used the cone-and-plate geometry. The experimental parameters were:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Stress (\sigma_0)</td>
<td>0.02 Pa</td>
</tr>
<tr>
<td>Final Stress (\sigma_f)</td>
<td>10 Pa</td>
</tr>
<tr>
<td>Sweep Time</td>
<td>600 s</td>
</tr>
<tr>
<td>Experimental Points</td>
<td>50</td>
</tr>
</tbody>
</table>

4.4.3.3 Determination of the Degree of Swelling for the Hydrogels
The degree of swelling, \(\alpha\), of the hydrogels was determined by weighing the dry hydrogels before and after immersion in the different chemical solvent media used in this study (distilled water, buffer solution at pH 7.0, and 0.05M NaCl solution). The degree of swelling is defined as:

\[
\alpha = \left\{ \frac{(\text{weight of hydrated gel}) - (\text{weight of dry gel})}{\text{weight of dry gel}} \right\} \times 100 \quad (4.11)
\]
4.5 Results and Discussion

Polyelectrolyte complex gels are prepared by mixing two oppositely charged polyelectrolytes; the network structure of polyelectrolytes complex gels is maintained by ionic cross-linking between polyelectrolytes; the gels are dispersed in water or in aqueous solutions. In this aspect, the charged state of functional groups of polyelectrolytes complex gels is considered to contribute to their network structure and can be function of several variables, as described below.

For the evaluation of the rheological properties of the polyelectrolyte complex gel (chitosan xanthan hydrogel), the following variables were analysed:

- Hydrogel concentration in solution (5, 6, 7, 8, 9, and 10% w/w).
- Chitosan used in the hydrogel formation:
  - Chitosan A: DA = 28%, $M_w = 8.2 \times 10^5$ g/mol, polydispersity = 4.7
  - Chitosan B: DA = 25%, $M_w = 1.08 \times 10^6$ g/mol, polydispersity = 3.1
- The range of the particle size in the dry hydrogel (125-75 μm and 75-55 μm).
- Chemical media used for the hydrogel dispersion (distilled water, buffer solution at pH = 7.0, and 0.05M NaCl solution).
- The waiting time (up to 390 minutes).

The rheological tests performed were:

a) Oscillatory-shear flow tests – Frequency sweep
   The complex moduli are measured at different frequencies. The frequency range is from 0.1 to 65 1/s.

b) Oscillatory-shear flow tests – Time sweep
   The complex moduli are measured at constant frequency (6.2832 1/s = 1 Hz and 62.832 = 10 Hz) and followed the behavior of the moduli with time (from 0 to 1000 seconds).

c) Steady-shear flow tests – Yield stress
   The strain is measured as function of the shear stress applied. The shear stress range is from 0.02 to 10 Pa.
The following features are observed, independently of the variables described above:

For oscillatory-shear flow tests:
- For the frequency sweep tests the storage modulus ($G'$) and loss modulus ($G''$) increase as frequency increases; while for the time sweep tests the complex moduli increase lightly as time increases.
- The complex viscosity ($\eta^*$) is frequency and time dependent; decreases as frequency increases and increases with time. The frequency range used (0.02 to 65 1/s) did not let us obtain near zero-shear-rate viscosity at low frequencies.
- $G'$ is always grater than $G''$ ($\eta^* > \eta'$); in hydrogels the elastic effects are more important than viscous effects. In general, it was very difficult to obtain data for the loss modulus ($G''$).

For yield stress tests:
- The strain ($\gamma$) is essentially independent of shear stress at very low shear stress; this permit us to determine the yield stress point which is the point where the strain begins to be dependent of the shear stress. From this point the strain increases with the shear stress.

These behaviors can be observed in figures 4.3 to 4.5, in which we show examples of the results obtained in laboratory. The plots correspond to a chitosan A/xanthan hydrogel with particle size range from 75 to 55 $\mu$m, dispersed in water at 25°C. In figure 4.3 is shown an example of the hydrogel behavior in a frequency sweep test. The system is 7.0% chitosan A/xanthan hydrogel concentration, without waiting time (that means that the hydrogel was dispersed in water and immediately was tested in the rheometer).

Figure 4.4 is an example of the hydrogel behavior in a time sweep test. The systems shown is 10.0% chitosan A/xanthan hydrogel concentration sample; the waiting time was 15 minutes and the test was performed at a constant frequency of 6.283 1/s (1 Hz). Figure 4.5 shows the hydrogel behavior in a yield stress test; the system shown is 8.0% chitosan A/xanthan hydrogel concentration sample without waiting time.
Figure 4.3 Storage modulus ($G'$) and complex viscosity as function of the frequency for 7.0% chitosan A/xanthan hydrogel with particle size range from 75 to 55 μm, dispersed in water, without waiting time and at 25°C. [Chitosan A: $M_w = 8.2 \times 10^5$, DA = 28%]

Figure 4.4 Storage modulus ($G'$) and complex viscosity as a function of the time for 10.0% chitosan A/xanthan hydrogel with particle size range from 75 to 55 μm, dispersed in water, with waiting time of 15 minutes and at 25°C. [Chitosan A: $M_w = 8.2 \times 10^5$, DA = 28%]
between 125 and 75 μm, a degree of swelling of 1609 means that this hydrogel can absorb water 16.09 times its weight. In other words, no free water will be present in hydrogels, where hydrogel concentration is 6.5% or higher.

Table 4.1 Degree of swelling and concentration range used for the systems studied.

<table>
<thead>
<tr>
<th>Hydrogel</th>
<th>Particle Size Range</th>
<th>α</th>
<th>Concentration range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chitosan A/Xanthan</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>125-75 μm</td>
<td>1609</td>
<td>7-10% w/w</td>
</tr>
<tr>
<td></td>
<td>75-55 μm</td>
<td>1521</td>
<td>7-10% w/w</td>
</tr>
<tr>
<td>Buffer Solution pH 7.0</td>
<td>125-75 μm</td>
<td>1270</td>
<td>7-10% w/w</td>
</tr>
<tr>
<td></td>
<td>75-55 μm</td>
<td>1162</td>
<td>7-10% w/w</td>
</tr>
<tr>
<td>0.05M NaCl Solution</td>
<td>125-75 μm</td>
<td>962</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>75-55 μm</td>
<td>1028</td>
<td>7-10% w/w</td>
</tr>
<tr>
<td><strong>Chitosan B/Xanthan</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>125-75 μm</td>
<td>2740</td>
<td>5-10% w/w</td>
</tr>
<tr>
<td></td>
<td>75-55 μm</td>
<td>2726</td>
<td>5-10% w/w</td>
</tr>
<tr>
<td>Buffer Solution pH 7.0</td>
<td>125-75 μm</td>
<td>536</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>75-55 μm</td>
<td>523</td>
<td>-</td>
</tr>
<tr>
<td>0.05M NaCl Solution</td>
<td>125-75 μm</td>
<td>1060</td>
<td>7-10% w/w</td>
</tr>
<tr>
<td></td>
<td>75-55 μm</td>
<td>1038</td>
<td>7-10% w/w</td>
</tr>
</tbody>
</table>

Notes: (α, the swelling index is defined in equation 4.11)

* The systems chitosan A/xanthan dispersed in 0.05M NaCl solution in the range from 125 to 75 μm was not studied.
- The systems chitosan B/xanthan dispersed in buffer solution need a minimum hydrogel concentration of 18%, concentration that it is out of our interest and for this reason were not prepared.

Figure 4.6 is an example of the concentration effect on the oscillatory-shear tests of chitosan-xanthan hydrogels at different concentrations. The example showed corresponds to chitosan B-xanthan hydrogel dispersed in water, with particle size range between 125-75 μm at 25°C. The waiting time was 45 minutes, and the hydrogel concentrations used were 5, 6, 7, 8, 9, and 10%. For all the frequency range, the storage modulus (G') increases when the hydrogel concentration increases too. When examining gels, no experimental evidence is given for G' to converge to a finite value for ω → 0, even when G' is only slightly dependent on frequency.
Figure 4.6 Storage modulus ($G'$) for chitosan B/xanthan hydrogel dispersed in water with particle size range between 125 and 75 μm, at different hydrogel concentrations and at 25°C. The waiting time was 45 minutes. [Chitosan B: $M_w = 1.02 \times 10^6$, DA = 25%]

Figure 4.7 Relationship between storage modulus ($G'$) and concentration for chitosan B/xanthan hydrogel dispersed in water with particle size range between 125 and 75 μm, at different hydrogel concentrations and at 25°C. Waiting time = 45 minutes. [Chitosan B: $M_w = 1.02 \times 10^6$, DA = 25%]
This graphic corresponds to chitosan/xanthan hydrogels dispersed in 0.05M NaCl solution, with particle size range from 75 to 55 μm at 25°C. The waiting time was 90 minutes.

The plots show how the storage modulus (G') increases with frequency; we observe different slopes. The relationship G' versus frequency for different chitosan samples also shows a crossover. For the case shown in figure 4.8 the frequency where the crossing of the lines occurs is a function of the hydrogel concentration. Table 4.2 shows the values of the crossover frequency for each hydrogel concentration.

<table>
<thead>
<tr>
<th>Chitosan/Xanthan Hydrogel</th>
<th>Hydrogel Concentration</th>
<th>Crossover Frequency (1/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>75-55 μm Dispersed in 0.05M NaCl solution</td>
<td>7.0%</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>8.0%</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>9.0%</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>10.0%</td>
<td>0.1</td>
</tr>
</tbody>
</table>

This value means that for a high crossover frequency is expected a low relaxation time for these systems.

\[ \lambda = 1 / \omega_c \]  \hspace{1cm} (4.12)

Where:
\[ \lambda \] Relaxation time (s)
\[ \omega_c \] Crossover frequency (1/s)

On a molecular scale the relaxation time of a polymer indicates the order of magnitude of time required for a certain proportion of the polymer chains to relax; that is, to respond to the external stress by the thermal motion. In the case of 7% hydrogel concentration, for example, the crossover frequency = 40 corresponds to systems that have a relaxation time approximately of 0.025 s; while the 10% hydrogel concentration has a relaxation time of 10 seconds. A complete set of crossover frequencies data were obtained only for the systems shown in figure 4.8; in general the crossover frequencies are out of the rheometer limits.
The possibility of working with hydrogels formed with two different chitosan samples has led to the knowledge of numerical values for the relaxation time as a function of the hydrogel concentration for systems with the same particle size range, dispersion media and temperature.

4.5.3 Effect of the Particle Size Range

After hydrogel formation we obtain a dry product that must be ground and sifted for better manipulation. We have used two different hydrogels fractions: the first has a particle diameter between 125 and 75 μm, and the second between 75 and 55 μm. The effects of the particle size on the rheological properties of the hydrogel are observed in figures 4.9 and 4.10. These plots correspond to chitosan A/xanthan hydrogel dispersed in water (figure 4.9) and dispersed in buffer solution pH 7.0 (figure 4.10) at 25°C. The waiting time was 90 minutes in each case.

In the case of water dispersions, we can observe at constant concentration that G’ is higher for the systems formed with the greater particle size. In the case of hydrogels dispersed in the buffer solution, we can observe at low concentration (7%) an opposite effect, but as soon as the concentration is increased the higher moduli correspond to the higher particle size. Also it is important to note that the differences due to particle size are less important in the case of dispersions in buffer solution; even at high hydrogel concentration there are values almost independent of the particle size effect.

Figures 4.9 and 4.10 also show that the effect of the particle size could be related to the nature of the dispersion medium used rather than the nature of the hydrogel. In the next section we will present how the effect of ionic strength in the system stabilises the electrostatic forces. This is important because differences due to particle size are less important in hydrogels dispersed in buffer solutions than in hydrogels dispersed in distilled water.

4.5.4 Effect of the Dispersion Medium and Waiting Time

In table 4.1 we presented the chemical media used to disperse the hydrogels: Distilled water, buffer solution at pH = 7.0, and 0.05M NaCl solution; all solutions have a pH = 7. The effect of these media on the rheology of the hydrogels was studied and as an example of the results obtained, shown in figure 4.11, exhibit a typical behavior. The plot corresponds to chitosan A/xanthan gel dispersed in the three different solvents used, for two hydrogel concentrations: 7.0
and 10.0%. The particle size range corresponds to the fraction with particle diameter between 75 and 55 µm, temperature = 25°C, and the waiting time = 90 minutes.

Figure 4.9 Storage modulus for chitosan A/xanthan hydrogel dispersed in water at two hydrogel concentrations (7 and 10%). Waiting time = 90 minutes and T = 25°C [Chitosan A: M_w = 8.2 x 10^5, DA = 28%]

Figure 4.10 Storage modulus for chitosan A/xanthan hydrogel dispersed in buffer solution at two hydrogel concentrations (7 and 10%). Waiting time = 90 minutes and T = 25°C [Chitosan A: M_w = 8.2 x 10^5, DA = 28%]
Figure 4.11 Storage modulus for chitosan A/xanthan hydrogel with particle size range between 75 and 55 μm, at different dispersion medium and two hydrogel concentrations (7 and 10%). Waiting time = 90 minutes and T = 25°C. [Chitosan A: \(M_w = 8.2 \times 10^5\), DA = 28%]

Figure 4.12 Storage modulus for chitosan B/xanthan hydrogel with particle size range between 125 and 75 μm, at different dispersion medium and four hydrogel concentrations (from 7 to 10%). Waiting time = 90 minutes and T = 25°C. [Chitosan A: \(M_w = 1.08 \times 10^4\), DA = 25%]
Figure 4.13 Storage modulus for chitosan A/xanthan hydrogel with particle size range between 125 and 75 μm, at different waiting times and T = 25°C. Hydrogel dispersed in water and buffer solution at concentration of 7.0%. [Chitosan A: $M_w = 8.2 \times 10^5$, DA = 28%]

Figure 4.14 Storage modulus versus waiting time at constant frequency ($\omega = 1.0$ 1/s) for chitosan A/xanthan hydrogel with particle size range between 125 and 75 μm and T = 25°C. Hydrogel dispersed in water and buffer solution at concentration of 7.0%. [Chitosan A: $M_w = 8.2 \times 10^5$, DA = 28%]
In this plot it is possible see how the storage moduli are always greater for the system dispersed in buffer solution as it was explained using figures 4.11 and 4.12. In addition, we can observe that the moduli range is wider in the case of the hydrogel dispersed in water, while for the hydrogel dispersed in buffer solution this range is very narrow, up to a point where the curves are almost superimposed. This can be explained by the effects of the counterions in a polyelectrolyte system: they decrease the strength of the electrostatic interactions by means of the salt addition. In the case of hydrogels dispersed in water, the bonds formed between the two polyelectrolytes and the water preserve the electrostatic interactions. This prevented us to attain constant moduli values at different waiting times.

From figure 4.13 we can also observe that G' decreases when we increase the waiting time. In general a hydrogel system recently prepared has higher moduli that a hydrogel that has been rheologically tested at longer times after its preparation. This effect is more pronounced in systems dispersed in water-only than in buffer solutions dispersions.

Figure 4.14 presents a comparison of the waiting times at constant frequency ($\omega = 1.0$ Hz) for the same systems showed in figure 4.13. We can observe how the waiting time is almost constant for the case where the hydrogel was dispersed in buffer solution; while for the hydrogel dispersed in water the moduli clearly decrease with increases on waiting time, especially at low waiting times. These effects observed with the waiting time are also observed in the time sweep oscillatory-shear tests. These results will be described in the next section.

4.5.5 Time Sweep Tests

Time sweep oscillatory tests are experiments that let us know the evolution of the rheological parameters with time at a constant frequency. In general, the effects observed in a frequency sweep test are the same as observed in a time sweep experiment. An example is presented in figure 4.15. This figure corresponds to the time sweep test (at constant frequency = 6.283 1/s = 1.0 Hz) for the same system studied in figure 4.6 when we presented the effect of the hydrogel concentration. The system is the chitosan B-xanthan hydrogel dispersed in water, with particle size range between 125-75 $\mu$m at 25°C. The waiting time was 120 minutes. The behavior observed is that storage modulus (G') increases when the hydrogel concentration increases for all the time values.
Figure 4.17 Storage modulus ($G'$) versus time at constant frequency ($\omega = 6.283$ 1/s) for chitosan A/xanthan hydrogel dispersed in different medium with particle size range between 75 and 55 $\mu$m at 25°C. Waiting time = 120 min. [Chitosan A: $M_w = 8.2 \times 10^3$, DA = 28%]

Figure 4.18 Strain versus shear stress for 9.0% chitosan A/xanthan hydrogel dispersed in buffer solutions with particle size range between 75 and 55 $\mu$m at 25°C. [Chitosan A: $M_w = 8.2 \times 10^3$, DA = 28%]
An important difference is that the effect of the time in the time sweep at constant frequency is almost nil. The curves are independent of the time and we can observe an unique value of $G'$ for each hydrogel concentration.

In figure 4.16 we plotted $G'$ versus concentration for the same systems described in figure 4.15 with data taken at 60 and 500 seconds. Two arbitrary times show the effect at short and long times. The results are almost equal at low concentrations; a small difference is observed when the hydrogel concentration is 10.0%, the point where elastic effects are more important.

As a final example of this type of tests, figure 4.17 presents the effect of the dispersion medium on the hydrogel in a time sweep test. The systems studied were the 7.0 and 10.0% chitosan A/xanthan hydrogel with particle size range between 75 and 55 $\mu$m at 25 °C. The time sweep was made at constant frequency of 6.28 1/s and the waiting time was 120 minutes.

The curves show the same result as observed in the frequency sweep test: the storage modulus is greater for systems that include some ionic strength (buffer solution and NaCl solution). The dependence of the moduli with the time is also almost insignificant in the first few seconds but for some systems can be important at larger times (nearly 1000 seconds after initiation the test). This can be explained by the elastic character of these systems but also for the possible evaporation effects presented in these tests.

4.5.6 Yield Stress Effect

There are materials that will not flow unless acted on by at least some critical shear stress, called the yield stress. The yield stress becomes a useful model property to describe the shear stress below which the flow of the materials becomes negligible.

In this study, yield stress tests were performed for the chitosan A-xanthan hydrogels with particle size range between 75 and 55 $\mu$m. The hydrogels were dispersed in the three solvents used in this study and the concentrations were from 7 to 10%, at 25 °C. As example of the results obtained, figure 4.18 presents the relationship between strain ($\gamma$) and shear stress ($\sigma$) for the case of hydrogel 9.0% in concentration and dispersed in buffer solution. We can observe that there is a low stress region where the strain is almost constant; while at higher shear stresses
the strain is a linear function of the stress. Then we can read an average value of the yield stress in the region where there is the junction of the constant-strain points and the stress-dependent strain points.

Table 4.3 Yield stress values as functions of the hydrogel concentration.

<table>
<thead>
<tr>
<th>Dispersion and Hydrogel</th>
<th>Medium Concentration</th>
<th>Yield Stress $\text{Pa}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water:</td>
<td>7% 8% 9% 10%</td>
<td>0.085 0.10 0.15 0.35</td>
</tr>
<tr>
<td>0.05M NaCl Solution:</td>
<td>7% 8% 9% 10%</td>
<td>0.09 0.15 0.40 0.60</td>
</tr>
<tr>
<td>Buffer Solution pH 7.0:</td>
<td>7% 8% 9% 10%</td>
<td>0.50 0.60 1.10 1.10</td>
</tr>
</tbody>
</table>

Table 4.3 shows the numerical values of the yields stress that we can read from the strain-stress relationships. In general the yield stress increases as concentration increases. This value is greater for the hydrogels dispersed in the buffer solutions, followed by the NaCl solutions dispersions, and finally from the water dispersions.

This behavior is similar to the phenomena explained in the previous sections. The storage modulus is a function of the hydrogel concentration. At higher concentration we expect higher $G'$ values. In the case of yield stress test we have for higher concentrations the critical shear stress is also higher.

With respect to the dispersion medium used, we can observe the same behavior as explained for figure 4.11, where the higher moduli were obtained with the systems with ionic strength. For the yield stress test we also have that the higher yields stress corresponds to the system that include phosphate ions, followed by the systems with chloride ions.
4.5.7 Comparison of the Rheological Properties between the Chitosan-Xanthan Hydrogels and Other Polysaccharide Gels

The chitosan-xanthan hydrogels are a special case of gels among the polysaccharide gels as they are formed by the complexation of natural polyions: the interaction of acidic polysaccharide (xanthan) and polycationic polysaccharide (chitosan). Only little rheological information exists for polysaccharide complexes formed by interactions anion-cation. However we can compare the rheological behavior observed in our system with the rheological properties that have been reported for polysaccharide-containing hydrogels like carrageenans, alginites, pectins or starch gels.

The carrageenan gels are one of the most rheologically-studied polysaccharide gels because of their dependence on ionic strength. The carrageenan gels are sulphated polysaccharide polymers that can be extracted from various genera of marine algae of the Rhodophyceae class. The carrageenans gels form strong gels in the presence of K\(^+\) or Ca\(^{++}\) ions but it has been observed that there is little gelation in the presence of Li\(^+\) or Na\(^+\) [CLARK et al., 1987].

Morris et al. [1980, 1981, and 1983] made shear modulus measurements on carrageenan gels and plotted modulus-concentration relationships for gels made in the presence of different cations. They also considered the effect of temperature. The rheological studies quantitatively confirmed the increase of gel rigidity (rigidity modulus, \(G_R\)) by K\(^+\) ions, but it also showed that these gels can be obtained in presence of Li\(^+\) or Na\(^+\) under high polymer and electrolyte concentrations. The values of the modulus are in the order of 10-20 Pa at low polymer concentrations. The modulus approaches 200 Pa for polymers gelled with K\(^+\) ions and 600 Pa for carrageenans gelled with Na\(^+\) ions. The lowest concentration studied is 0.25% and the highest 3.0% by weight.

The rigidity of these hydrogels can be conveniently compared with the moduli values obtained in our systems at very low frequency. In other words we can compare the rigidity modulus (\(G_R\)) of the carrageenans with our storage modulus (\(G'\)) at low frequency.
With our chitosan-xanthan hydrogels we obtain $G'$ values comprised between 8 and 120 Pa (see Figure 4.6). These values contrast with those of the carrageenans. Using lower quantity of material in the system we could obtain more rigid gels. The precise gelation properties depend on the cations present. We must take in account another important difference: our chitosan-xanthan hydrogels are irreversible gels, while the carrageenans gels undergo conformational changes with temperature by means of coil-helix (order/disorder) transitions [ROSS-MURPHY, 1995].

Strong and weak gels are classified on the basis of their mechanical spectra. The typical strong gel spectrum, over the frequency range of $10^{-2}$ to $10^{2}$ rad/s consists of two nearly horizontal straight lines. $G'$ is typically 1-2 orders of magnitude grater than $G''$, and both may show some slight increase at higher frequencies. The weak gels show mechanical spectra that are similar but no identical; the profiles of both moduli show a slight frequency dependence with no sign of a Newtonian plateau at low frequencies with $G'$ (the energy storage modulus) exceeding $G''$ (the energy dissipation modulus) at all frequencies.

Chitosan-xanthan hydrogels can be considered as weak gels because their $G'$ is always function of frequency (at low and high frequencies) with no evidence of a Newtonian plateau. However rheological results for the chitosan-xanthan hydrogels can be compared with the rheological moduli of strong gel such alginites gels.

Alginates are salts of alginic acid and occur as intercellular material in brown algae. Their function as structure-forming components appears to result from their capacity to gel in the presence of certain divalent cations. Unlike the carrageenans gels, the alginites do not form thermoreversible gels. But, like the carrageenan gels, the rheological properties of the alginate gels are function of the cation used which has an influence on gel strength.

Segeren et al. [1974] reported the rheological properties for the system Ca$^{2+}$-alginate gels (1.4% w/w). As in the case of chitosan-xanthan hydrogels, the dynamic analysis is restricted to the storage modulus $G'$ because the ratio $G''/G'$ is lower than 0.01; $G''$ has minimal influence in the complex modulus. The $G'$ values reported for these alginate gel are slightly higher than 1,000 Pa and are essentially constant over all the frequency range ($10^{-1}$ to $10^{1}$ rad/s).
as C', with n comprised between 7 (high degree of polymerization) and 4.4 (low degree of polymerization); this behavior has been related with high elasticity of the amylose gels.

Pectins constitute another category of polysaccharide gels. Pectins are substances that originate from plants, consisting of the partial methyl esters of polygalacturonic acid, are water soluble, and are able to form gels under suitable conditions, by temperature changes or by the introduction of specific counterions and pH variations [LAPASIN, 1995]. Commercial pectins are divided into high ester (HM) pectins and low ester (LM) pectins according to the degree of methyl esterification (see Section 4.3.1.2). The G' modulus of LM-pectin gels is 10 times that of G'' and independent of frequency over a wide frequency range, which indicates that the solid character is predominant as in a strong gel. An increase in temperature leads to a sharp decrease in G' [DURAND et al., 1990]. Watson [1966] found in HM-pectin gels the presence of plastic flow, that means the existence of a elastic limit; the highest level of stress which a material is capable of sustaining without any permanent deformation. In our hydrogels at the tested conditions the yield stress determination show that there is no of plastic behavior; it behaves as an elastic material.

We can also compare our results with the behavior of other weak gels. The comparison is possible with xanthan. At concentrations higher than 1% it behaves almost like a gel. Since xanthan is a polyelectrolyte its rheological properties would be expected to change in response to changes in ionic strength. As in our systems there are an important relationship between the ionic strength and the concentration. In the case of the chitosan-xanthan hydrogels the dissolution in electrolyte medium increases the viscosity and moduli. In the case of xanthan alone at low concentration (0.15% w/w) the addition of an electrolyte, such as NaCl, reduces viscosity slightly; it behaves as a solution and can be related to our results for chitosan solutions. However at higher xanthan concentrations, the presence of an electrolyte has the opposite effect: it behaves like a weak gel. The moduli and viscosity increase with the ionic strength similar to chitosan-xanthan hydrogels [PETTITT, 1982].

For 1.0% w/w xanthan with 0.02M KCl, a weak gel behavior was observed, where it was not possible to obtain G' independently of the frequency. The values of the moduli show G' ~ 10^2 and G'' ~ 10^1 [LAPASIN et al., 1995]. The values of G' are similar to the values obtained for the chitosan-xanthan hydrogels but the difference is that the G'' values are near to those of G' and
5. RHEOLOGICAL MODELING FOR CHITOSAN/XANTHAN HYDROGELS

5.1 Summary

The rheological modeling of the chitosan/xanthan hydrogels results presented in the previous chapter is discussed in this chapter. For modelling the oscillatory-shear results we used the weak gel model of Gabriele et al. [2001]. In this model a “weak gel” is assumed to coincide with the “critical gel” of Winter’s theory, but given the flow nature of our systems a finite relaxation time is assumed. The model supposes that the material structure is represented by a cooperative arrangement of flow units.

As a function of the model parameters obtained we can establish relationships between the number of interlinked rheological flow units in the three-dimensional structure and the network strength. We found from these parameters that the experimental variables affecting the rheological characteristic were the hydrogel concentration, the dispersion media used, and the degree of polymerisation of chitosan used in the hydrogel formation.

5.2 Rheological Models in Physical Hydrogels: Literature Review

Rheological modeling is important in the design of flow processes, in quality control related to storage and processing stability and in predicting textures of solutions, colloids, gels and other complex materials. Rheological modeling in gels becomes particularly useful when predictive relationships can be developed, starting from the molecular architecture of the constitutive species.

The following aspect of the rheological behavior of physical gels are discussed with particular emphasis on recent investigations:

- Viscoelastic models
- Yield Stress models
- Molecular models
5.2.1 Viscoelastic Models for Physical Gels

Gels, such as all polymer systems, that show viscoelastic behavior, as reviewed in Chapter 4. To completely characterise a viscoelastic material such as hydrogels, it is necessary to express the results of stress relaxation and creep experiments using mechanical viscoelastic models. These models are convenient ways of representing the data and can shed light on the time scale of the molecular mechanisms contributing to their viscoelastic response.

When dealing with structured materials as gels and colloidal systems, the most relevant and useful theory is probably the Winter theory (section 4.2.3.3). This theory characterises gels and provides some useful “tools” to evaluate, with a good approximation, the “gel point” where liquid-solid (sol-gel) phase transition occurs, using simple rheological tests. When biological hydrogels are considered, it is experimentally observed that dynamic moduli are often almost linear and slightly parallel in a frequency range that typically ranges between 0.1 Hz and 100 Hz. The linearity displayed suggests that in the time scale considered hydrogels behave as critical gel according to Winter’s theory.

To predict the suggested flow character, Gabriele et al. [2001] assumed that the hydrogels are constituted by flowing units interacting with one another to an extent that might induce breakup. During dynamic oscillatory experiments the hydrogel may be considered as a combination of flow units where:

\[ G^* = A_F \omega^{\nu_f} \]  \hspace{1cm} (5.1)

With \( z \) being the number of rheological units interacting with one another in the three-dimensional structure. \( A_F \) represents the gel strength and may be interpreted as the “strength” of the interaction between those units. This simple approach can give useful information about the rheological structure of some materials characterised by a three dimensional network in which particles are linked by more or less strong interactions. This kind of approach was applied to different gels and suspensions such as jams, dough and yoghurt [GABRIELE et al., 2001].

In the linear viscoelastic regime, discrete or generalised Maxwell models generally describe stress relaxation curves quite well. The resulting behavior is typical of a viscoelastic liquid. For
\[ \tau = \frac{\tau_0}{\left(1 + p(n-1)\right)^{\frac{1}{n-1}}} \]  

(5.5)

Where \( n \) is a parameter derived from the power-law dependence of internal viscosity. High values of \( n \) are practically associated with solid-like properties. Another empirical equation proposed by Peleg [1980] that could describe the solid-like and liquid-like behaviors is represented by:

\[ \tau = \tau_0 \frac{k_1 + (k_2 - 1) t}{k_1 + k_2 t} \]  

(5.6)

Where \( \tau_0 \) is the initial stress, \( k_1 \) governs the initial rate of decay and \( 1/k_2 \) represents the asymptotic level of the relaxation parameter (see equation 5.7). The parameters can depend on the time scale of the initial straining stage, and in such cases they offer a simple tool to evaluate, experimentally, the magnitude of such effects. For \( k_2 > 1 \), the equation yields the asymptotic value of the stress when \( t \) tends to \( \infty \), whereas a viscoelastic liquid should be characterised by \( k_2 = 1 \). If the equation could hold for a sufficiently long time, \((1 - 1/k_2)\) would represent the equilibrium stress, typical of the solid-like behavior. The equation has been fitted to stress-decay curves of several polysaccharide gels, and examples of the hydrogel systems modeled are the agar gel (5%) [POLLACK et al., 1980] and gellan gels (0.5-2.5%) [NUSSINOVI\( \check{\text{s}} \) et al., 1990].

Several constitutive models have been used to explain data for hydrogels and suspension systems. The Bird-Carreau and the Doi-Edwards models presented in section 3.2.4 are examples. The semi-empirical Bird-Carreau model facilitates the estimation of parameters and is easily applicable to a variety of materials such as concentrated dispersions of polysaccharides [KOKINI et al., 1987], protein networks [DUS et al., 1990; COCERO et al., 1991], dispersions of guar polysaccharide, wheat gluten, and wheat flour doughs [KOKINI, 1994]. The Doi-Edwards model does not currently permit prediction of the rheological properties of complex mixtures, but it nevertheless provides major clues in designing biological molecules with desired rheological properties. Kokini [1994] used this model to correlate the rheological properties of 5% apple pectin dispersions.
The viscoelastic properties of gels and dispersions have frequently been demonstrated by relaxation curves. An ideal mathematical representation of a relaxation phenomenon is based on a minimum of constants with meaningful physical information and on equation sensitive to physical changes in the system but insensitive to arbitrary parameters in a simple form as possible.

To apply these conditions to relaxation curves different mathematical procedures have been tested. The relaxation curves have been normalised and the decaying parameter \( Y(t) \) was calculated as follows [PELEG, 1979; PELEG et al., 1983]:

\[
Y(t) = \frac{F_0 - F(t)}{F_0}
\]  

(5.7)

Where \( F(t) \) is the force recorded after \( t \) minutes of relaxation and \( F_0 \) is the initial force. Because during relaxation the deformation is maintained constant, the parameter \( Y(t) \) represents the decay of the force, stress or moduli related to variation of \( F(t) \).

Typical shape of the function \( Y(t) \) versus time suggests the simplified mathematical form of [MICKELEY et al., 1957]:

\[
Y(t) = \frac{abt}{1 + bt}
\]  

(5.8)

Where \( a \) and \( b \) are constants. The meaning of "a" in the present context is related to the level of stress decay during relaxation (\( a = 0 \) means the stress does not relax at all, \( a = 1.0 \) means the stress level eventually reaches zero). The constant "b" is representative of the rate at which the stress relaxes (\( 1/b \) is the time necessary to reach the level of \( a/2 \)).

Relaxation curves and models of agar gels (5%), apple solid, cheddar cheese, and bologna sausage obtained under various deformation conditions were presented by Peleg [1979] and showed that in all the tested materials relaxation curves are well represented by equation 5.8.
- Non-linear stationary viscoelasticity: The stationary solution for the transient network model of reversibly crosslinked gels is found under arbitrary macro-deformation. For shear flow with constant shear rate, the number of active chains, shear-viscosity, first and second normal stress differences are calculated as functions of shear rate, and these properties depend rather sensitively on a chain breakage function.

- Dynamic mechanical moduli: Linear response to oscillatory deformations is studied for a model transient network made up of uniform polymer chains reversibly crosslinked by associating end groups. The dynamic mechanical moduli are obtained as functions of the frequency and the chain breakage rate.

- Time-dependent phenomena: Stress relaxation following a sudden macro-deformation is calculated for several realistic models of the chain breakage rate. On large time scales, stress decay obeys a power law.

### 5.3 Some Considerations about Hydrogels Rheology and Oral Drug Delivery

The cationic character of the chitosan clearly provides particular possibilities for utilisation in controlled-release technologies. Chitosan-based hydrogels are highly swollen, hydrophilic polymer networks that can absorb large amounts of water and drastically increase in volume. Also chitosan has anti-acid and anti-ulcer activities that prevent or weaken drug irritation in the stomach. Chitosan-based formulations appear to float and gradually swell in acid media [GUPTA et al., 2000].

#### 5.3.1 Oral Drug Delivery

Oral administration of drugs is one of the simplest and safest. However an oral controlled-release formulation is subjected to frequent changes of environment during its transit through the gastrointestinal tract as it passes from strongly acidic to weakly alkaline media in the lower part of the small intestine. This and other factors combine to introduce variability in the performances of oral controlled delivery systems [FELT et al., 1998].

The absorption of a drug is dependent upon the physicochemical properties of the drug, the nature of the drug, and the anatomy and physiological functions of the sites responsible for drug absorption.
Anatomically, the swallowed drug rapidly reaches the stomach. Eventually, the stomach empties its contents into the small intestine. A delay in the gastric emptying time for the drug to reach the duodenum will slow the rate and possibly the extent of drug absorption. Some factors influencing gastric emptying are volume, osmotic pressure, physical state of gastric contents (chemicals and drugs), and miscellaneous factors (body position, viscosity, emotional states, etc.).

5.3.2 Rheology of Intestinal Fluid Flow

Drug delivery requires the mixing and translocation of medicaments within the intestinal lumen. Propulsive forces that are generated primarily by the musculature of the intestine induce this movement. Since the musculature can only apply a force through a distance by changing its geometry, the geometry of the intestinal tube varies with time as intestine moves its content.

Intestinal fluid flow and wall motions are linked by hydraulic forces. This linkage makes it impossible to consider the production and control of fluid flow independently of the origin and control of intestinal wall motion or vice versa. The physical principles that govern the intestinal mobility are mechanics of fluid flow and wall motions [MACAGNO et al., 1981; WEEMS, 1987].

* Fluid Flow: Two types of forces are associated with the study of fluid flow, body and surface forces. Surface forces are those forces acting on the boundaries of a fluid by direct contact with the surrounding environment. The displacement of fluid in space by the constriction of a region of intestine involves the action of surface forces. External forces are normal stresses referred as the internal pressure of the fluid.

The fluid will be deformed continuously when subjected to shearing stress, and this deformation can be represented by the generalised Newtonian fluid models frequently used in rheology. The major difficulties confronting the application of these equations to intestinal fluid flow is the lack of a formal understanding of the internal viscous stresses. The application of surface force to the fluid is complex because the geometry of the intestine is not fixed but varies with time in a complicated fashion [WEEMS, 1987].
* Wall Motions: In the absence of precise kinematic description of intestinal wall motion, fluid mechanical models are used to determine the flow in a conduit of varying geometry by simple periodic functions [MACAGNO et al., 1981]. The wall motions modeled are usually limited to simple periodic waveforms that are axisymmetric and peristaltic (propagate along the axis of the tube or channel).

We have considered that fluid flow and wall motions are governing the physical interaction of intestinal mobility. Wall and fluid motions would result as the geometry of the intestinal region changed to establish new equilibrium states between surface forces and stresses within the intestinal wall. Finally it is important to note that the overall control of intestinal fluid flow results from interaction between internal and external control systems. The internal systems include control cells within the intestinal tube. The external systems include the central nervous system and the endocrine system [WEEMS, 1987].

5.4 Results and Discussion

5.4.1 Model Description: Gabriele et al. Weak Gel Model [GABRIELE et al., 2001]

The understanding of rheological phenomena is based on two theories: the phenomenological theory that clarifies the stress-strain-time relationship, and the molecular theory that provides evidence of the molecular origin of viscoelastic behavior. For synthetic polymers, both theories have been successfully applied. However, biological systems, which are regarded as "heterogeneous dispersions", exhibit considerable variability in their rheological properties because of their complexity, so that the application of the classical rheological models is limited [ZOON et al., 1990].

Many weak gels and complex dispersions can be treated as three-dimensional networks where weak interactions ensure the stability of the structure. In structured materials such as gels, the most relevant theory is probably the Winter theory, see Section 4.2.3. This theory characterises gels and gives some useful tools to evaluate with a good approximation the gel point. Winter found that at the gel point not only the longest relaxation time diverges but also the relaxation modulus follows a power law [WINTER, 1989]:

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\[ G(t) = At^{-n} \quad (5.10) \]

Where:

- \( n \) Relaxation exponent
- \( A \) Gel strength

The relation is valid for \( \lambda_0 < t < \infty \), where \( \lambda_0 \) is a characteristic time for crossover to small scale dynamics.

Equation 5.10 can be transformed for the case of oscillatory tests at fixed frequency, \( \omega \), by:

\[ G(\omega) = A\omega^n \quad (5.11) \]

For \( 0 < \omega < 1/\lambda_0 \). This critical gel is assumed to correspond to the beginning of a solid and therefore it relaxes in an infinite time and does not flow, the viscosity being infinite.

In our case, weak gels and dispersions behave like a three-dimensional network, but they also flow. The linearity displayed suggests that weak gels behave like a critical gel as in Winter’s gels theory but must be considered as a gel-like networks capable of flowing because they show a finite relaxation time.

Gabriele et al. weak gel model suggests that the structure of a material corresponds to a cooperative arrangement of flow units, forming a strand that interacts to a certain extent. The model introduces the coordination parameter \( z \), that is the number of flow units interacting with one another to give the observed flow response of the material. The model suggests that the extent of the interactions is measured by \( z \).

During dynamic oscillatory experiments the gel strands may be considered as a combination of flow units where \( z \) is the number of rheological units interacting with one another in three-dimensional structure. The system can be:

\[ G^*(\omega) = \sqrt{G'(\omega)^2 + G''(\omega)^2} = A_f \omega^\frac{1}{z} \quad (5.12) \]
$A_F$ is the gel strength, and can be interpreted as the strength of the interactions between those units.

Gabriele et al. model is a simple relationship but gives a qualitative idea of the link between microscopic and macroscopic properties of physical gels. This approach will give us useful information about the rheological structure of chitosan/xanthan hydrogels, considering the existence of a three-dimensional network in which flow units are linked by more or less weak interactions.

5.4.2 Oscillatory-Shear Flow Modeling

For the oscillatory-shear experimental results the Gabriele et al. model has been used for the storage modulus ($G'$) curves. In equation 5.12 we can see that the model is for the complex modulus ($G^*$). However, in our case it was not always possible to obtain adequate values for the loss modulus ($G''$). It was too small, near to zero, as described in Chapter 4. Since the loss moduli are much lower than the storage moduli, we can approximate the model as:

$$G'(\omega) = A_F \omega^{\frac{1}{z}}$$

(5.13)

Some examples of these curves are presented in figures 5.1 and 5.2. Figure 5.1 corresponds to chitosan A/xanthan gels dispersed in the three different media used, for 7.0 and 10% hydrogel concentration; the particle size range corresponds to the fraction with particle diameter between 75 and 55 \( \mu \)m, temperature = 25\(^\circ\)C and waiting time = 90 minutes.

Figure 5.2 corresponds to chitosan B/xanthan hydrogels dispersed in two different media, for 7.0 and 10.0% hydrogel concentration; the particle size range correspond to the fraction with particle diameter between 125 and 75 \( \mu \)m, temperature = 25\(^\circ\)C and waiting time = 90 minutes. For both figures the symbols correspond to experimental data and the lines to the model. These plots show that the model fits for the data independently of the hydrogel concentration, dispersion media, particle size range and other experimental features. Table 5.2 and 5.3 show the Gabriele et al. model parameters for the chitosan/xanthan hydrogels studied; $A_F$ has units of (Pa s\(^{1/z}\)) and $z$ is dimensionless.
Figure 5.1 Storage modulus for chitosan A/xanthan hydrogel with particle size range between 75 and 55 µm, at different dispersion media for 7.0 and 10.0% hydrogel concentration. Waiting time = 90 minutes and T = 25°C. [Chitosan A: $M_w = 8.2 \times 10^5$, DA = 28%]

Figure 5.2 Storage modulus for chitosan B/xanthan hydrogel with particle size range between 125 and 75 µm, at different dispersion media for 7.0 and 10.0% hydrogel concentration. Waiting time = 90 minutes and T = 25°C [Chitosan B: $M_w = 1.02 \times 10^6$, DA = 25%]
Table 5.2 Gabriele et al. model parameters for the chitosan A/xanthan hydrogels

<table>
<thead>
<tr>
<th>Particle Size Range</th>
<th>Dispersion Media</th>
<th>Hydrogel Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>7.0%</td>
</tr>
<tr>
<td>125-75 μm</td>
<td>Water</td>
<td>A_F = 47.1</td>
</tr>
<tr>
<td></td>
<td>z = 5.82</td>
<td>z = 7.68</td>
</tr>
<tr>
<td></td>
<td>Buffer Solution</td>
<td>A_F = 87.6</td>
</tr>
<tr>
<td></td>
<td>z = 8.42</td>
<td>z = 6.77</td>
</tr>
<tr>
<td>75-55 μm</td>
<td>Water</td>
<td>A_F = 25.3</td>
</tr>
<tr>
<td></td>
<td>z = 3.73</td>
<td>z = 4.18</td>
</tr>
<tr>
<td></td>
<td>Buffer Solution</td>
<td>A_F = 126.2</td>
</tr>
<tr>
<td></td>
<td>z = 7.48</td>
<td>z = 5.88</td>
</tr>
<tr>
<td></td>
<td>0.05 NaCl Solution</td>
<td>A_F = 56.5</td>
</tr>
<tr>
<td></td>
<td>z = 5.48</td>
<td>z = 5.92</td>
</tr>
</tbody>
</table>

Table 5.3 Gabriele et al. model parameters for the chitosan B/xanthan hydrogels

<table>
<thead>
<tr>
<th>Particle Size Range</th>
<th>Dispersion Media</th>
<th>Hydrogel Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5.0%</td>
</tr>
<tr>
<td>125-75 μm</td>
<td>Water</td>
<td>A_F = 2.9</td>
</tr>
<tr>
<td></td>
<td>z = 1.76</td>
<td>z = 2.61</td>
</tr>
<tr>
<td></td>
<td>0.05 NaCl Solution</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>z = 3.76</td>
</tr>
<tr>
<td>75-55 μm</td>
<td>Water</td>
<td>A_F = 4.7</td>
</tr>
<tr>
<td></td>
<td>z = 2.02</td>
<td>z = 2.24</td>
</tr>
<tr>
<td></td>
<td>0.05 NaCl Solution</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>z = 3.52</td>
</tr>
</tbody>
</table>

Figure 5.3 shows the behavior of the gel strength, A_F, for the case of chitosan B/xanthan hydrogel dispersed in water and 0.05M NaCl solutions, for both particle size fractions. In general for all hydrogels studied, the gel strength (A_F) increases with an increase in hydrogel concentration, the values being always lower than for the systems dispersed in water. The parameter z also increases with an increase in hydrogel concentration, but only in the case of
chitosan B/xanthan hydrogels. For the case of chitosan A/xanthan hydrogels the numerical values of $z$ are higher than for chitosan B/xanthan hydrogels, but the changes of the $z$ parameter with respect to the concentration are not regular.

![Figure 5.3 Gel strength parameter from Gabriele et al. model versus hydrogel concentration for the chitosan B/xanthan hydrogels dispersed in water and 0.05M NaCl solutions and for both particle size ranges (125-75μm and 75-55μm). Waiting time = 90 minutes, $T = 25^\circ C$ [Chitosan B: $M_w = 1.02 \times 10^6$, DA = 25%]]

The results reveal that the final structure and the final properties of these hydrogels are function of a great number of factors, and that there are interactions between these factors that must be considered in order to obtain hydrogels with specific properties. From the analysis of the parameter values reported in tables 5.3 and 5.4 we can conclude that the model reveals that the gels are sensible principally to three experimental factors: hydrogel concentration, dispersion media, and chitosan used in the hydrogel formation.

- Effect of the concentration: Gel strength, $A_F$, increases with increasing concentration. This is confirmed by the oscillatory tests where $G'$ increases continuously over the entire concentration range. This means that independently of the number of interactions ($z$ value) the systems turns out to be stronger when concentration increases.
• Effect of the dispersion media: Water has a lower value of $z$ that means a lower number of interactions and also a lower value of $A_F$. This means smaller networks strength with respect to the values reported for systems dispersed in NaCl and buffer solutions. When compared to similar systems dispersed in NaCl and buffer solutions the differences in $z$ are not important and this means a similar number of interactions. However the interactions in the systems dispersed in buffer solutions are stronger (higher values of $A_F$).

• Effect of the chitosan: In general, chitosan A-based hydrogels present $z$ values higher than the values obtained for chitosan B-based hydrogels. It reflects that a higher number of interactions were formed when a chitosan with lower molecular weight was used, possibly due to a steric effect.

For the systems based on chitosan A, an increase of the network strength (due principally to concentration increases) is followed by an increase of the network connectivity. For chitosan B-based hydrogels an increase of the network strength (due to concentration effects) is almost independent of the network connectivity.

As a conclusion on the chapters about the rheology of chitosan/xanthan hydrogels: wide ranges of experimental conditions were studied. They provided clear indications on the experimental variables that are important for the rheology of our hydrogels. The results show that calculations of the storage modulus ($G'$) could be successfully performed by the model chosen. The storage moduli calculated from the Gabriele et al. model were similar as those obtained from oscillatory experiments. The parameters obtained have a well-defined physical meaning and provide an understanding about the nature and behavior of the chitosan-xanthan hydrogels. Once again, all the results for the experiments and models with hydrogels are included in an appended document (CD disc: AgustinMartinez_RheoChitoxan_1997-2001).

In the following chapter we will present the conclusions for this thesis and we will propose a direction of the work that we think will complement the work done in this thesis.
6. CONCLUSIONS

This study has investigated the rheological properties of chitosan solutions and chitosan/xanthan hydrogels. Experimental results and modeling are presented and discussed.

Chitosan solutions have been found to behave like non-Newtonian polymer solutions, showing a low-shear rate Newtonian plateau and a high-shear rate non-Newtonian region. Oscillatory-shear tests have shown that the dynamic moduli increase with frequency. The loss modulus ($G''$) has been shown to be higher than the storage modulus ($G'$), indicating that viscous effects are more important than elastic effects in chitosan solutions (no crossover point in the experimental range studied). The normal forces and the primary normal stress difference increase with the shear rate (as is expected for polymeric systems).

The temperature effect was primarily to increase chitosan solubility at higher temperatures, which led to a more Newtonian flow behavior. It has also been observed that as the chitosan concentration increases intermolecular interactions start to appear and higher resistance to flow is observed.

The rheology of chitosan solutions is also a function of the type of acid used for chitosan dissolution. The steady-shear viscosity and the complex moduli have been found to be lower for solutions in hydrochloric acid than for solutions prepared in weak aqueous acids, indicating a strong interaction between chitosan and hydrochloric acid. The effect of the acidic solutions used and the presence of ionic strength is explained by the different neutralization effect of the protonated amino group, as well as steric and electrostatic effects.

The most important parameter has been found to be the salt addition, especially in systems for which weak acids have been used to dissolve chitosan. At high chitosan concentrations (2%) the viscosity and moduli are almost the same for systems with and without salt. However, when the chitosan concentration decreases differences between the viscosity and moduli of the solution with and without salt were observed for systems in which chitosan was dissolved in weaker acid solutions. The presence of a counterion led to lower moduli than without counterion. However the counterion effect was observed to be small when chitosan
was dissolved in hydrochloric acid. The results reflect that the presence of a counterion changes the density of the intermolecular physical entanglements principally in dilute chitosan solutions. When hydrochloric acid is present, the effect of an excess number of chloride ions on the viscosity and moduli indicates a complete suppression of electrostatic repulsions and for this reason the rheological properties remain almost constant.

Hydrogels formed by complexation from chitosan and xanthan have been rheologically characterized by oscillatory shear measurements under small-deformation conditions. The complex viscosity and the moduli are frequency dependent, with a small increase of the moduli and decrease of the complex viscosity as frequency increases. G' is always greater than G'', which means that in chitosan/xanthan hydrogels the elastic effects are more important than viscous effects. For each frequency range, the storage modulus (G') increases with the chitosan/xanthan hydrogel concentration.

Higher moduli were obtained in systems with ionic strength: buffer solution and sodium chloride solution instead of systems dispersed only in distilled water. In solvents with ionic strength, higher moduli correspond to cases where the ion present in the medium is the phosphate anion. This can be explained via the electrostatic equilibrium changes induced by the counterions; the ionic strength modifies the internal structure of the hydrogel and this effect is observed as an increase of the moduli and the viscosity of the hydrogel. It also showed a decrease in G' at low “waiting time”, but G' reaches an almost constant value at high “waiting time”. The “waiting time” has been defined as the time between the moment when the hydrogel is dispersed and the moment when the rheological test is performed started.

The rheological experiments also include yield stress determination using a steady-shear method that measures the hydrogel deformation at different shear stresses. At very low shear stress the strain of the hydrogels was almost independent of the shear stress. This permitted to determine the yield stress. We found that higher yield stresses correspond to systems that have ionic strengths such as phosphate ions and chloride ions. This is the same effect observed for moduli values as function of the ionic strength.

Modeling of the hydrogels rheological properties was done using the Gabriele et al. model, which was established for weak gel systems. The model parameters obtained are dependent
on the hydrogel concentration, dispersion medium used, and the type of chitosan used in the hydrogel.

The structure of the hydrogel system is explained as a combination of flow units with parameters related to the number of rheological units correlated with one another in a three-dimensional structure and to the strength of the interactions between the flow units. The results reveal that the final structure and the final properties of these hydrogels are functions of varying factors (such as hydrogel concentration, and nature of the dispersion medium), and that interactions between these factors must be considered in order to prepare hydrogels with desired properties.

As a summary, the objectives described in Chapter 1 have been achieved a complete description of the rheological properties in chitosan solutions and chitosan/xanthan hydrogels. We have also developed empirical relationships and applied rheological models that relate the microstructural characteristic of the systems with its macroscopic properties. For chitosan solutions the rheological behavior was successfully described in only a few master curves, while for hydrogels the parameters obtained can be used to envision the behavior of these systems when used for drug-delivery vehicles.

As far as recommendations for future work in this area we would suggest the following:

For chitosan solutions,
- The effect of the chitosan characteristics (degree of acetylation, molecular weight and polydispersity) in the rheological properties.
- The effect of the ionic strength could be studied with more precision via modification of the salt used and variation of the ionic strength concentration.

For chitosan/xanthan hydrogels,
- The temperature effect is the most important variable that must be taken into account. For this purpose it will be important to develop adequate rheological tests that control or prevent water evaporation from the hydrogel.
- Development of tests that provide relaxation curves for these hydrogels. Stress growth or creep experiences could be sufficient to obtain the relaxation parameters.
- For oral drug delivery applications, rheological measures with variations of pH could be interesting because the hydrogel will be subjected to different pH stages when introduced into the human body.
- For pharmaceutical applications the effect of the addition of specific drugs over the rheological properties should be of interest.
7. REFERENCES


