RHEOLOGICAL CHARACTERIZATION OF HYDROXYPROPYL METHYLCELLULOSE SOLUTIONS: GELATION AND MOLECULAR INTERACTIONS WITH ADDITIVES

by

Carlos Alberto Pinzón De la Rosa

A dissertation submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

In

Chemical Engineering

UNIVERSITY OF PUERTO RICO
MAYAGÜEZ CAMPUS
2015

Aldo Acevedo, Ph.D. President, Graduate Committee	Date
Ubaldo Córdova, Ph.D. Member, Graduate Committee	Date
Patricia Ortiz, Ph.D. Member, Graduate Committee	Date
Rafael Méndez, Ph.D. Member, Graduate Committee	Date
Carmen Vega Ph.D. Representative of Graduate Studies	Date
Aldo Acevedo, Ph.D. Chairperson of the Department	Date

ABSTRACT

The discovery and development of highly hydrophobic drugs represents an additional challenge for the pharmaceutical industry, as traditional methods of encapsulation, administration, and dosage are not always appropriate. Physically formed edible films have been proposed as a solution to this problem due to their ability to encapsulate and stabilize these particles.

Many methods have been proposed for the characterization, and improvement of the physical and morphological properties of these films. However, many of these methods are subjective and time-consuming. In this study, it is proposed to evaluate the effect of the addition of various additives, such as flavorants, plasticizers, and two highly hydrophobic BCS (biopharmaceutical classification system) class II drugs, through rheological characterization of the gelation temperature of the precursor solutions.

The evaluation of the addition of flavorants with different functional groups in their structure, and the effect of their concentrations in the gelation temperature of polymer solutions was studied. Gelation was affected by the polymer relative solubility, and the interactions between polymer and organic part of the solvent mixture which depends on the functional groups present in the flavorant molecules. These results could be applied in the estimation of the effect of the addition of small soluble molecules with similar chemistry.

The ability of the polymer to stabilize these hydrophobic particles through van deer Walls and hydrophobic interactions with the surface of the particles was studied. Results showed a relation between the polymer-particle affinity and the gel formation mechanism. A synergistic effect caused by the polymer molecular weight contribute to the amount of hydrophobic interactions that the polymer can make with other drug or polymer molecules, affecting the flexibility of the system.

The effect of the addition of plasticizers to the mixture was evaluated. HPMC solutions with glycerol were further studied because it has been reported that at high concentrations of the plasticizer, phase separation may occur. A combination of steady-state rheology measurements of the polymer solutions and NIR-CI were used to study the relation between solution properties and films morphology. During the film casting process, the evidence of phase separation is measured by NIR-CI and optical images.

RESUMEN

El descubrimiento y desarrollo de fármacos altamente hidrófobos representa un desafío para la industria farmacéutica, ya que los métodos tradicionales de encapsulación, administración, y dosificación no siempre son apropiados. Membranas comestibles se han propuesto como una solución a este problema debido a su capacidad para encapsular y estabilizar estas partículas.

Muchos métodos se han propuesto para la caracterización y mejora de las propiedades físicas y morfológicas de estas películas. Sin embargo, muchos de estos métodos son subjetivos y consumen mucho tiempo. En este estudio se propone evaluar el efecto de la adición de varios aditivos, como saborizantes, plastificantes, y dos drogas hidrofóbicas BCS tipo II, a través de la caracterización reológica de la temperatura de gelación de las soluciones precursoras.

Se estudió la evaluación de la adición de saborizantes con diferentes grupos funcionales en su estructura, y el efecto de sus concentraciones en la temperatura de gelación de las soluciones de polímero. La gelación se vio afectada por la solubilidad relativa del polímero, y las interacciones entre polímero y parte orgánica de la mezcla de solvente que depende de los grupos funcionales presentes en las moléculas saborizantes. Estos resultados podrían ser aplicados en la estimación del efecto de la adición de pequeñas moléculas solubles con química similar.

Se estudió la capacidad del polímero para estabilizar estas partículas hidrófobas a través de fuerzas de van deer Walls e interacciones hidrofóbicas con la superficie de las partículas. Los resultados mostraron una relación entre la afinidad del polímero y la partícula, y el mecanismo de formación del gel. Un efecto sinérgico causado por el peso molecular del polímero contribuye a la cantidad de interacciones hidrofóbicas que el polímero puede hacer con otras moléculas de droga o de polímero, lo cual afecta la flexibilidad del sistema.

Se evaluó el efecto de la adición de plastificantes a la mezcla. Soluciones de HPMC con glicerol se estudiaron más a fondo, ya que se ha reportado que a altas concentraciones del plastificante, puede ocurrir separación de fases. Una combinación de mediciones reológicas en estado estacionario y NIR-CI se utilizaron para estudiar la relación entre propiedades de la solución y la morfología de las membranas. Durante el proceso de fundición de la película, la evidencia de separación de fases se comprobó por NIR-CI e imágenes ópticas.

To God, thank you for never let me down

To my Family in Colombia, and extended family in Puerto Rico, for never losing the faith in me

To Doriliz, my wife, my love and my life
Thank you for always been there for me, I Love You so much!

ACKNOWLEDGMENTS

First I would like to thank to my thesis advisor Dr. Aldo Acevedo for his support, guidance and patience during this project. I also extend my gratitude to my committee members, Dr. Rafael Mendez, Dr. Patricia Ortiz and Dr. Ubaldo Cordova, for their constructive collaboration and input in this work.

I would like to thank to Vivian Florian, Ana Cameron, Mariel Santiago and other lab-mates for their friendship and help when needed. Also would like to thank to Dr. Rodolfo Romañach and his students, for allowing me to use their facilities to perform my experiments.

Thanks to the Engineering Research Center for Structured Organic Particulate Systems (ERC-SOPS), for the financial support and the opportunity to share my work.

And last but no least I would like to say thank you, to my wife, my love, for all the unconditional love and support you gave my during all these years, for being there when I needed you and for encourage me to be a better person every day.

`

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION AND BACKGROUND

The discovery of new, powerful and water insoluble drugs represents a challenge for researchers to develop new and novel drug dossing and delivery methods.[1, 2] Films of natural polymers with a suspended active pharmaceutical ingredient (API) have been proposed as feasible delivery system.[3, 4]

The use of biopolymers in the pharmaceutical and food industry is not new. These materials have been widely used in varied pharmaceutical applications such as binders, emulsifiers, suspending and disintegrating agents, and coating materials in encapsulation processes.[5-7] Additionally these materials have been used in packaging and edible film coatings for food preservation.[1, 2]

Polymers from natural sources are in many cases a better choice than synthetic polymers since they can be degraded in a short period of time by living microorganisms or in the human digestive system, avoiding environmental contamination or accumulation of polymers in the body, respectively. Some examples of biopolymers commonly used in the pharmaceutical industry are polysaccharides, such as starch and cellulose, and proteins such as casein, collagen and keratin.[8, 9]

These materials are capable of forming physical and/or chemicals gels, which could provide the capacity for encapsulation and immobilization of particles making them

attractive for drug delivery applications. Many of these materials are cheap, renewable, its processing can be performed in existing polymer processing technology, and many of them are approved for human consumption by the U.S Food and Drug Administration agency (FDA).[10-12]

Some advantages of these types of delivery methods include easy and continuous processing, fast bioavailability, easy dossing, long term stability, and less formulation excipients.[3, 4] However, other effects may be evaluated in the formulation process to avoid negative changes in the structural properties of the films. The addition of particles to the films it is conditioned to certain maximum load, this is because a high concentration of particles in the films may affect the mechanical properties [13], also particle (additives)-polymer interactions may affect the gelation process.[14]

Other additives, such as plasticizers, surfactants, and flavorants, may be incorporated in these systems to provide stabilization, flexibility and flavor masking.[15, 16] Nevertheless, molecular interactions may be present affecting the morphological, mechanical, and functional properties of the resulting films. The interactions between the surfactants, flavorants, plasticizers, and the structural gelling polymer may affect the dispersion of the particles, form aggregates, or weaken the polymer matrix preventing effective gel formation.

1.1 Biopolymer gels

Polymer gels are colloidal systems in which the polymer molecules are physically or chemically cross-linked in a tridimensional network and the space between molecules is mostly filled with a liquid solvent, making them suitable for particle encapsulation applications. These networks exhibit no flow when in the steady-state and behave primarily as a solid system.[17]

A detailed gel classification system was proposed by Flory,[18] who proposed a structural criteria classification of these. The four main types of structures identified are:

I. Well-ordered lamellar structures: including gel mesophases. In this category systems like soap gels, clays and phospholipids can be found. Electrostatic interactions and van der wall forces are the primary interaction forces.

II. Covalent polymeric networks: completely disordered. In this category, systems such as vulcanized rubber, phenolic resins, and paint films can be found. Chemical reactions, chemical cross-linking, and covalent bonds are the main gel forming interactions.

III. Polymer networks formed through physical aggregation. In this category, systems predominantly disordered, but local ordered zones can be observed such as gelatin, gellan gum or agarose gels. Gelation in these systems is controlled by crystallization, hydrogen bonding, van der wall forces and hydrophobic interactions.

IV. Particulate: disordered structures. Some examples of systems in this category are flocculent precipitates and aggregated proteins. Main interactions found in these systems can be classified as: antibody-antigen interactions and specific interaction between well-defined sites of the proteins.

For simplification, gel systems can also be divided into two main categories according to the cross-linking method of the polymer chains, which are physically or chemically cross-linked systems.[19]

Chemical gels are networks formed through covalent bonds induced by added reagents (cross-linkers) or between the polymer molecules (reacting monomers), by a series or reactions such as condensation (critical percolation), addition (kinetic growth) or vulcanization (cross-linking). These systems fall into the second category according to the classification system proposed by Flory.

Physical or pseudo-gels are networks formed through physical aggregation, predominantly disordered, but with regions of local order.[20] These systems are formed by weak associative forces such as hydrogen bonds, van der wall forces or hydrophobic interactions; they are induced mainly by changes in thermodynamic parameters in the medium as pH, salt concentration (ionic), or temperature (thermotropic).

1.2 Physical gelation of biopolymers

Physical gels are characterized by dynamic cross-links that are constantly created and destroyed, changing its state between solid and liquid under influence of environmental factors. This restructuring ability makes them an important class of materials with many applications, such as drug delivery agents,[21] tissue engineering[22-24], and optical devices.[25]

Classical gelation theory developed by Flory [26] and Stockmayer [27] in the early 1940's, considers the linking process of the polymer chains through random bonds, but ignores the rate of creation/destruction of them which introduce an additional degree of freedom. Consequently, the stress of these materials cannot be determined only by its deformation, but also by the continuous creation/destruction of the network, the density of cross-links, and their spatial organization.[20]

As stated before, physical gels are formed by weak associative forces induced by changes in the medium. A group of gels that can be formed by changes in the temperature (thermotropic) are of special interest due to the potential for use as templates for drug delivery applications. Many of these thermotropic polymers are able to form thermoreversible networks, which have the ability to change its structure with changes in temperature. Gelatin for example is a biocompatible polymer with many applications in both the food and pharmaceutical industry.[28, 29]

These thermoreversible networks are formed through physical interaction between polymer chains such as crystallization, helix formation, or complex formation. Flory in his work describes the structure formation of these networks as shown on Figure 1.

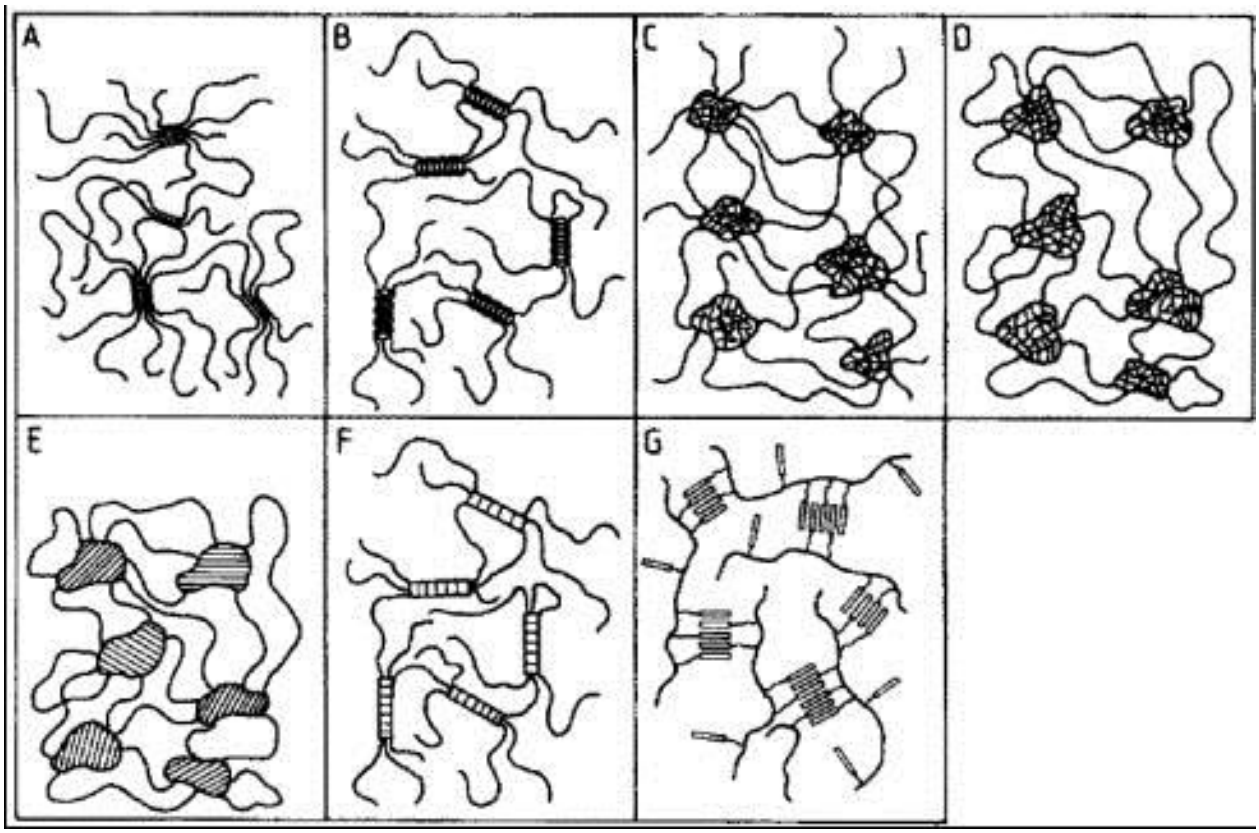


Figure 1. Schematic view of various kinds of thermoreversible physical gels: A PVC/plasticizer; B aqueous gelatin; C atactic PS in CS2; D triblock copolymer SDS in tetradecane; E PO-EO-PO triblock copolymer in water; F s-PMMA and i-PMMA in toluene; G dissolved SCLCP. Taken from [30]

where A represents gel formation by miscellar cristallites, helix formation is represented in B, C, D and E represents networks formed by phase separation, F represents a complex formation and finally G represents a gelation process caused by interaction between mesogenic groups in the main chain and/or in the side chain.

In this work, a biopolymer matrix capable of forming thermoreversible physical gels was characterized. The selected polymer was hydroxypropyl methylcellulose (HPMC), a derivative of cellulose in which the hydroxyl groups are replaced by hydroxypropyl and methyl groups [31. 32] by reacting wood or cotton cellulose fibers with propylene oxide and methyl chloride in the presence of caustic soda. This polymer was chosen by its ability to form physical gels when heated, providing a template that could be used for drug (particle) encapsulation. According to the Flory classification system, this is a type III gel, and the gelation is controlled by crystallization, hydrogen bonding, van der wall forces and hydrophobic interactions.

1.3 Thermoreversible polymer gels characterization methods

The understanding of the physical properties of thermoreversible biopolymer solutions is of great importance for the development of processing methods and applications. For pharmaceutical applications, the ability to encapsulate particles in a gel network has been widely studied recently[33, 34], since these polymer matrixes could be used as a more efficient delivery method for active pharmaceutical ingredients (APIs).[35]

The concentration of particles incorporated in a colloidal system has a significant effect on its microstructure; other important factors able to affect the mechanical and morphological properties of these systems are the surface chemistry and temperature.[36] Gelation temperature (T_{gel}) is a material property that can be related to changes in the

morphological and mechanical properties of biopolymer films, additional to that it have great importance because it can be used to set processing conditions.[37]

The addition of APIs to the polymer system could affect the gelation process since polymer-particle (or particle-particle) interactions may occur, decreasing the selectivity of the actives sites in the polymer chain. T_{gel} is evaluated as a measure of how the addition of these particles (APIs) affects the stability of the gel network. This is the temperature at which the system stops behaving like a liquid and start behaving more like a solid due to the formation of a tridimensional network at the macroscopic scale (bulk).

Other properties may be relevant to the process, such as viscosity, yield stresses, thixotropy, turbidity, elastic properties, etc., but in this work the main effort was to determine how the incorporation of additives such as plasticizers, flavorants, and drug particles (APIs) affect the microstructure of the system by measuring the T_{gel}.

There are many techniques to determine gelation temperature in polymers, which include optical [38], mechanical [39], and thermal methods. Medina-Esquivel and collaborators used a simple optical method to determinate T_{gel} of aqueous solutions of agar when heating. A non-coherent light source was placed in front of the sample and the transmitted light was measured with a photodiode. Since the transmitted light decays while the sol-gel transition occurs, the gelation temperature can be approximated. [40] However, this

method only works with systems that get cloudy or opaque when the gelation is occurring and is limited when the system is opaque in nature.

In 2002, Chung and collaborators reported two simple mechanical methods to estimate the gelation transition temperature of PLGA-g-PEG aqueous solutions.[41] The first method consisted on placing the sample on a test tube and heating at a rate of 0.2°C/min, the test tube was inverted and the criterion of flow or no-flow was used to determine the gelation temperature. The second method consisted on placing a steel ball in the solution and measure the time period for the ball to fall 5 cm when increasing the temperature, the sol-gel transition was determined by extrapolating two slopes observed in a temperature vs travel time diagram (see Figure 2A). A modification of the Stokes' law for yield stress fluids can be used to determinate the sol-gel transition,[42] using the specific weight of the ball, the specific weight of the fluid, the diameter of the sphere, and the velocity of the sphere, and extrapolating the two slopes observed in a dynamic viscosity vs temperature graph (see Figure 2B). It is clear that the particle size and density dictate the balance between gravitational stress and the yield stress. These methods revealed good results compared to rheological measurements, however a variation of 1-2°C was reported and the measurements were time consuming.

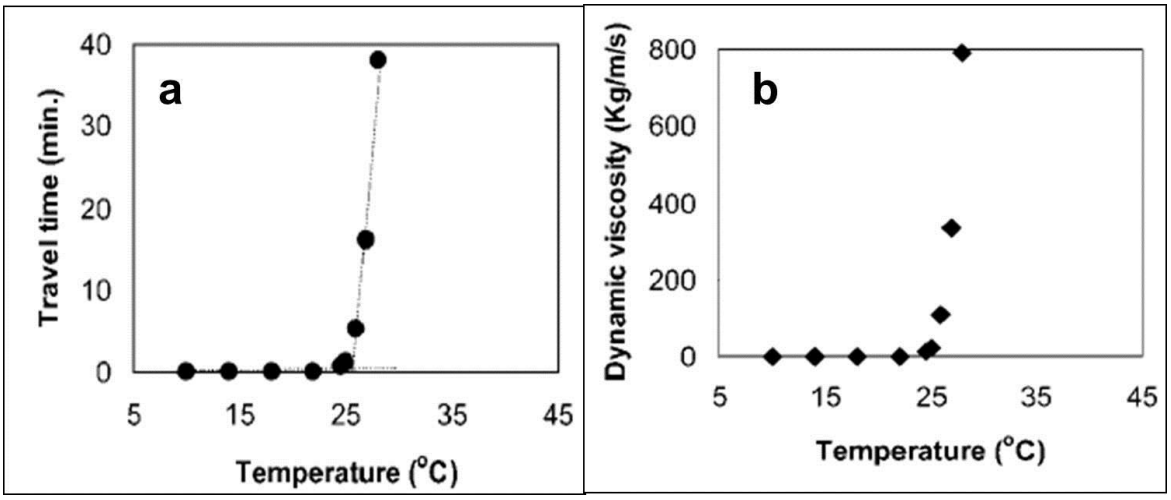


Figure 2. Determination of sol-to-gel transition temperature of PLGAg-PEG copolymer (I) aqueous solution (29 wt %) by extrapolation of two lines from a curve describing the falling time of steel ball over 5 cm as a function of temperature. Travel time (a) and dynamic viscosity (b). Reproduced from [41]

Other methods as turbidimetry, NIR spectroscopy, and dynamic light scattering have also have been reported.[43-45] However, turbidimetry can only be used on clear samples and is subjective to the opacity of the sample; NIR-spectroscopy is a very time consuming technique and is hard to work with liquids, and finally DLS does not provide structural information of the system due to cluster interactions during the gelation process.

The most common methods for T_{gel} determination are based on rheological measurements, this is mainly because these methods are not subjective to optical properties of the sample or theoretical approximations, and the sample requires almost no pretreatments. The use of these methods has been reported extensively in literature. Boudhani and collaborators used oscillatory measurements of G' and G" while heating a PVC plastisol (PVC + plasticizer)

sample, a constant frequency and heating rate (0.5°C/min) was maintained during the process.[46] In their work they report three different temperatures T_{Gmin}, T_c and T_{Gmax}, during the gelation process. T_{Gmin} is the temperature at which the moduli values are minima, characterizing the onset of the gelation process.

Rheological measurements have been demonstrated to be very useful in the characterization of macroscopic properties of polymer solutions. But have also been reported to be useful to describe the gelation process at the nanoscale. Barrera and collaborators used cobalt ferrite nanoparticles coated with a PEG-silane to determine the gelation temperature of gelatin solutions, by measuring the out-of-phase component of the complex susceptibility of the nanoparticles when suspended in the polymer solution.[47] The results showed that this method is effective to determine T_{gel}, but a slight difference was observed in comparison with results obtained from macroscopic rheological methods, which was attributed to interactions between the particles and the network due the size of the nanoparticles.

For bulk or macro-rheological measurements, two rheological tests have been extensively used for the gelation temperature determination through the observation of sol-gel transitions; constant stress temperature ramp (CSTR) viscosity method and oscillatory dynamic test.[48, 49] The first method is based on the determination of an overshoot or discontinuity at the sol-gel transition while the sample is cooled (or heated) at constant stress. The gelation temperature is determined by the intercept of the two tangents

observed in the curve,[50] as depicted on the Figure 3. This figure shows a solution of HPMC E4M 2% with added naproxen (1%) which was subjected to a temperature ramp at a constant stress. The viscosity was monitored during the process and the gelation temperature was determinate as the intercept of the two tangent lines.

The second rheological method is the oscillatory dynamic method, which consists in monitoring the storage and loss moduli (G', G''), as function of temperature as shown in Figure 4. The gel point (T_{gel}) is assumed to correspond to the temperature where G' = G''. This is an approximated value of T_{gel}, since it has been shown that for some chemical gels the gelation starts at a much lower temperature.[46]

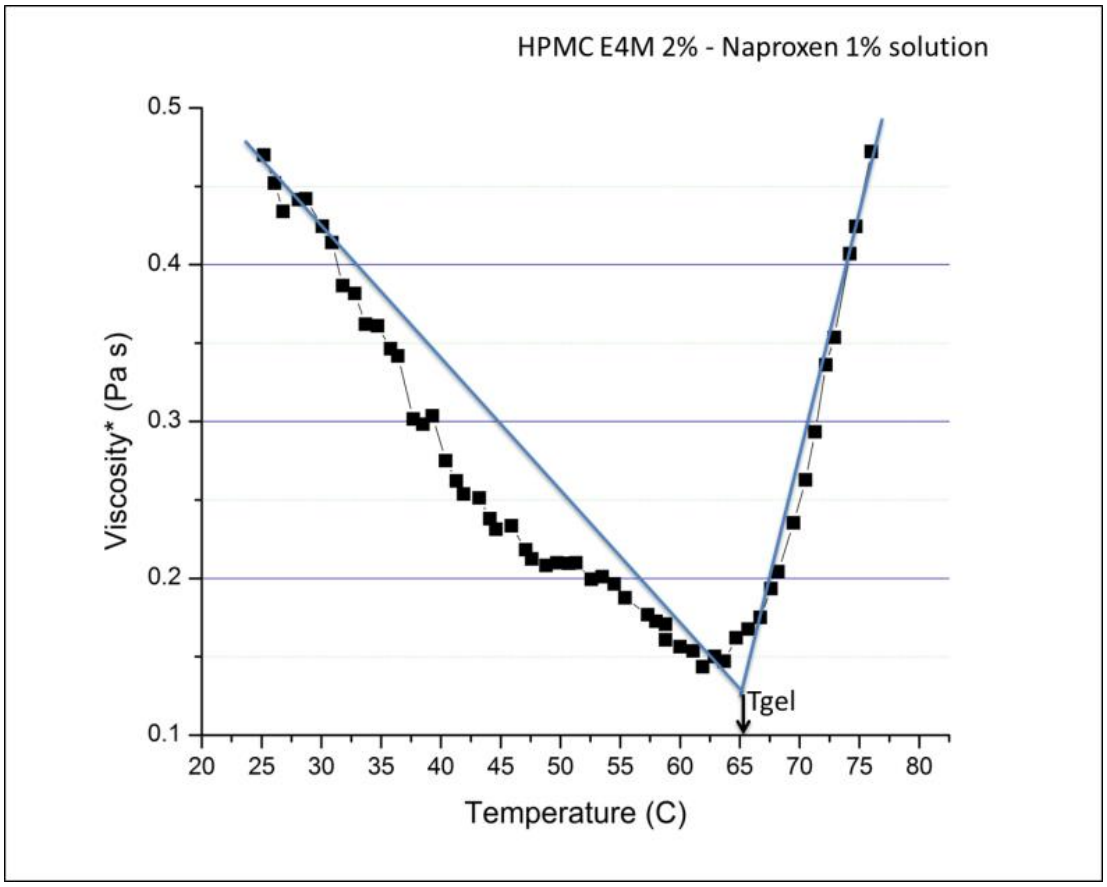


Figure 3. Constant stress temperature ramp (CSTR) viscosity method. T_{gel} determination. HPMC E4M 2% - Naproxen 1%

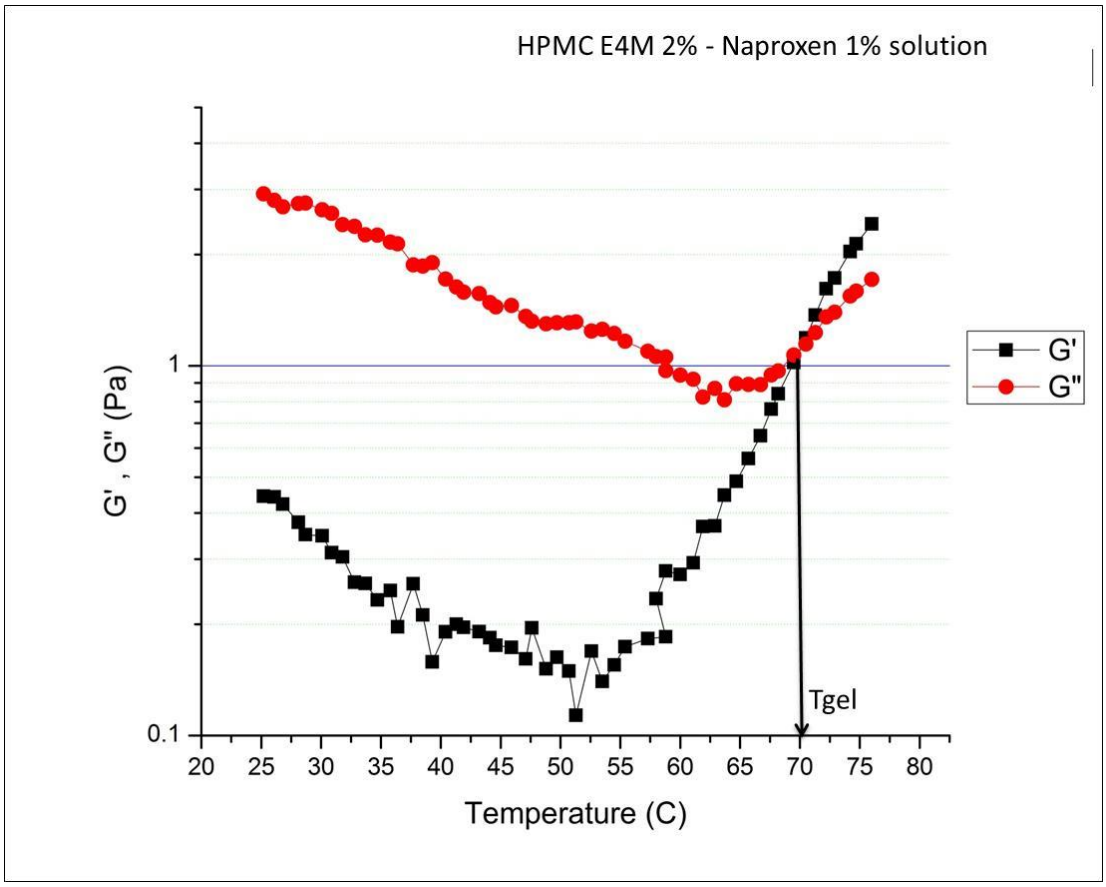


Figure 4. Oscillatory dynamic method. T_{gel} determination. HPMC E4M 2% - Naproxen 1%

These methods are based on the work of Winter and Chambon, where they reported that G' and G" are proportional to ω^n, where ω is the frequency and n is the slope of the plot G'G" vs ω (in logarithmic scale). For n it can be found values of 1/2, -3/2, -7/2, -11/2, etc. but the solution is only valid for n=1/2.[51] However, this method despite being more accurate is more time consuming, therefore the approximation of G'=G" is generally accepted.

In this work solutions of HPMC (E4M and E15LV viscosity grade) will be evaluated in the presence of different API's at different concentrations, flavorants, and plasticizers, for the determination of the gelation temperature, and how the presence (or absence) of the different additives affect the gelation process, the oscillatory dynamic method was used for the T_{gel} determination.

1.4 Hydroxypropyl methylcellulose

The proposed polymer matrix to be studied in this work is a hydrophilic, non-ionic semisynthetic biopolymer known as hydroxypropyl methylcellulose or HPMC (Figure 5), derived from cellulose which reacts with a mixture of methylene chloride and propylene oxide.[52]

This biopolymer is used in several applications. In the pharmaceutical industry it is used in ophthalmic applications like lubricant, moist dispersions for contact lenses and artificial tears.[53-55] Also, HPMC is used as excipient in many oral medicaments and in drug delivery systems.[56, 57]

This biopolymer is selected due to its ability of form physical gels when solubilized in water and heated to certain temperature.[58] The polymer provides the capacity for particle encapsulation that is desired for the suspension of particles. HPMC is a cheap, biodegradable, abundant, and easily processed polymer for gel applications. In the

structure of the HPMC, the hydroxyl (ROH) group of a cellulose molecule has been substituted for hydroxypropyl or methyl groups.[59] It is widely accepted that the dominant phenomenon in the gelation process of HPMC is the intermolecular association of hydrophobic groups in the polymer chains, leading to cross-linking and gel formation. In our special case, this phenomenon occurs when the polymers is heated.[60]

There are two main stages in the gelation process of HPMC, initially when molecules are subjected to heat an increase in their energy is reflected in an increasing of their mobility, exposing their hydrophobic regions and allowing that these regions interact forming aggregates and finally a gel network. However, Suzuki and collaborators proposed that both hydrophobic interactions and hydrogen bonds are related to gel formation of methylcellulose solutions.[61]

In accordance to the previous statement, Haque and collaborators reported that there are two ‘waves’ of structure formation within the thermal gelation process of methyl substituted cellulose esters, such as methylcellulose and hydroxypropyl methylcellulose. First ‘wave’ correspond to a partial melting of structures present in solution, reversible on cooling with a significant hysteresis. In this stage the polymer chains formed in bundles starts to separate, exposing the hydrophobic substitutions, in this stage is also reported the formation of water 'cages' structures around hydrophobic substituents exposed to the aqueous environment. The second ‘wave’ or stage, correspond to the disruption of these cage-like structures around the hydrophobic substitutions, and then the following

hydrophobic interactions between the exposed hydrophobic groups on the polymer chains. Both stages are characterized by increases in the storage moduli G'.[62]

An inhibition in the gelation of highly hydroxypropyl substituted cellulose esters is observed, compared with methylcellulose polymers, and it can be explained, at least partially, by the more polar (less hydrophobic) character of the hydroxypropyl groups compared with the methoxyl substituents. An inhibitory effect on chain packing (compared with methylcellulose) at low temperatures is likely to arise from a combination of the internal flexibility ok the hydroxypropyl group and its physical size, which may be difficult to accommodate within a close-packed aggregate. At higher temperatures the same resistance of the hydroxypropyl groups to incorporation within ordered structures, is a limiting factor for the gelation process.[31]

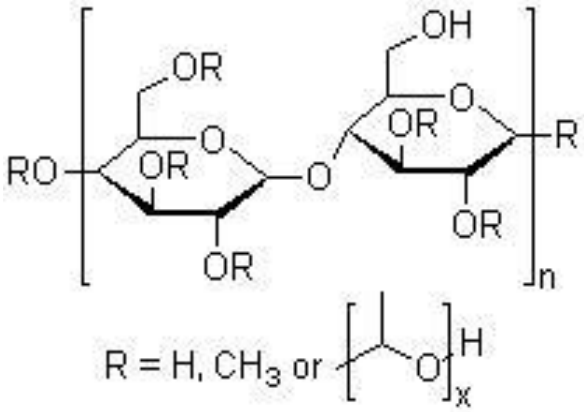


Figure 5. HPMC Molecule Representation

The behavior of hydrogels during the thermogelation process can be substantially altered by the addition of different additives such as salts.[63] As hydrogen bonds and other intermolecular interactions play and important role in the gelation process, it is expected that when another molecules such as flavorants are added in the system the gelation process may be affected, either by hydrogen-hydrogen interactions or any other change in the properties of the suspending medium that may inhibit the gelation process.

In this work is proposed the study of the effect of the addition of different formulation parameters in the gelation temperature of hydroxypropyl methylcellulose solutions, in order to understand deeply the molecular interactions between polymer and additives that may affect the morphology and mechanical properties of thin edible films for drug delivery applications.

1.5 References

1. Schuetz, Y., Jordan R., Olivier R. A Novel Thermoresponsive Hydrogel Based On Chitosan. European Journal Of Pharmaceutics And Biopharmaceutics 68(1) (2008); 19-25.
2. Wagh V., Samant M. Polymers Used In Ocular Dosage Form And Drug Delivery Systems. Asian Journal Of Pharmaceutics 2(1) (2008); 12 - 17.
3. Vondrak B., Barnhart S. Dissolvable Films For Flexible Product Format In Drug Delivery. Pharmaceutical Technology (2008); 20 – 28.
4. Renuka M., Avani A. Formulation Development Of Taste-Masked Rapidly Dissolving Films Of Cetirizine Hydrochloride. Pharmaceutical Technology (2009); 48 – 56.
5. Vegi G., Srinivasan P., Beedu S., Khard R., Diwan P. Emulsifying Properties Of Gum Kondagogu (Cochlospermum Gossypium), A Natural Biopolymer. Journal Of The Science Of Food And Agriculture 89 (2008); 1271 - 1276.
6. Laovachirasuwan P., Srijesdaruk V., Chitropas P., Otsuka M. The Physicochemical Properties Of A Spray Dried Glutinous Rice Starch Biopolymer. Colloids And Surfaces B: Biointerfaces 78 (2010); 30 - 35.
7. Bajaj I., Saudagar P., Singhal R. Gellan Gum: Fermentative Production, Downstream Processing And Applications. Food Technology And Biotechnology 45(4) (2007); 341 - 354.
8. Belcarz A, Ginalska G., Zalewska J., Rzeski W., Sarczyk A., Kowalczuk D., Godlewski P., Niedzwiadek J. Covalent Coating Of Hydroxyapatite By Keratin Stabilizes Gentamicin Release. Journal Of Biomedical Materials Research Part B: Applied Biomaterials August (2008).
9. Friess W. Collagen – Biomaterial For Drug Delivery. European Journal Of Pharmaceutics And Biopharmaceutics 45 (1998); 113–136.
10. Reppas C., Swidan S., Tobey S., Turowski M., Dressman J. Hydroxypropylmethylcellulose Significantly Lowers Blood Cholesterol In Mildly Hypercholesterolemic Human Subjects. European Journal Of Clinical Nutrition (2009); 63, 71–77.
11. Baldrick P. The Safety Of Chitosan As A Pharmaceutical Excipient. Regulatory Toxicology And Pharmacology 56 (2010); 290–29.
12. Kogan G., Soltes L., Stern R., Gemeiner P. Hyaluronic Acid: A Natural Biopolymer With A Broad Range Of Biomedical And Industrial Applications. Biotechnology Letters (2007); 29:17–25.
13. Casariego A., Souza B., Cerqueira M., Teixeira J., Cruz L., Díaz R., Vicente A. Chitosan/Clay Films' Properties As Affected By Biopolymer And Clay Micro/Nanoparticles' Concentrations. Food Hydrocolloids 23 (2009); 1895–1902.
14. Morris E., Nishinari K., Rinaudo M. Gelation Of Gellan - A Review. Food Hydrocolloids 28 (2012); 373-411.
15. Moonprasith N., Sa-Nguanthammarong P, Kongkaew C., Loykulnant S. Effect Of Surfactant On Gelation Of Hydroxypropylmethyl Cellulose In Skim Natural

Rubber Latex Serum. Journal Of Metals, Materials And Minerals. 18 - 2 (2008); 89 - 91.
16. Rodrıguez M., Oses J., Ziani K., Mate J. Combined Effect Of Plasticizers And Surfactants On The Physical Properties Of Starch Based Edible Films. Food Research International 39 (2006); 840–846.
17. Ferry J. Viscoelastic Properties Of Polymer Solutions. Part Of The Journal Of Research Of The National Bureau Of Standards 41 (1948) 53–62.
18. Flory P. Introductory Lecture. Faraday Discussions Of The Chemical Society 57 (1974); 7-18.
19. Hellio D., Djabourov M. Physically And Chemically Crosslinked Gelatin Gels. Macromolecular Symposia 241 (2006); 23–27.
20. An Y., Solis F., Jiang H. A Thermodynamic Model Of Physical Gels. Journal Of The Mechanics And Physics Of Solids 58(12) (2010); 2083–2099.
21. Fuertes I., Caraballo I., Miranda A., Millán M. Study Of Critical Points Of Drugs With Different Solubilities In Hydrophilic Matrices. International Journal Of Pharmaceutics 383 (2010); 138–146.
22. Yong Lee K., Mooney D. Hydrogels For Tissue Engineering. Chemical Reviews 101(7) (2001); 1869–1879.
23. Wang Q., Wang L., Detamore M., Berkland C. Biodegradable Colloidal Gels As Moldable Tissue Engineering Scaffolds. Advanced Materials 20 (2008); 236–239.
24. Niknamasl A., Ostad S., Soleimani M., Azami M., Salmani M., Lotfibakhshaiesh N., Ebrahimi-Barough S., Karimi R., Roozafzoon R., Ai J. A New Approach For Pancreatic Tissue Engineering: Human Endometrial Stem Cells Encapsulated In Fibrin Gel Can Differentiate To Pancreatic Islet Beta-Cell. Cell Biology International 38(10) (2014); 1174–1182.
25. Chen Y., Hirnschall N., Findl O. Comparison Of Corneal Wetting Properties Of Viscous Eye Lubricant And Balanced Salt Solution To Maintain Optical Clarity During Cataract Surgery. Journal Of Cataract And Refractive Surgery 37(10) (2011); 1806–1808.
26. Flory P. Molecular Size Distribution In Three Dimensional Polymers III. Tetrafunctional Branching Units. Journal Of The American Chemical Society 63(11) (1941); 3096–3100.
27. Stockmayer W. Theory Of Molecular Size Distribution And Gel Formation In Branchedchain Polymers. The Journal Of Chemical Physics 11(2) (1943); 45–55.
28. Lou X., Chirila T. Swelling Behavior And Mechanical Properties Of Chemicallly Cross-Linked Gelatin Gels For Biomedical Use. Journal Of Biomaterials Applications 14 (1999); 184–191.
29. El-Malah Y., Nazzal S. Hard Gelatin And Hypromellose (HPMC) Capsules: Estimation Of Rupture Time By Real-Time Dissolution Spectroscopy. Drug Development And Industrial Pharmacy 33 (2007); 27–34.
30. Te Nijenhuis K. Introduction Chapter 1. Thermoreversible Networks – Viscoelastic Properties And Structure Of Gels. Springer Books. (1997).

31. Haque A., Richardson R., Morris E. Thermogelation Of Methylcellulose. Part II: Effect Of Hydroxypropyl Substituents. Carbohydrate Polymers 22 (1993); 175-186.
32. Sarkar N. Thermal Gelation Properties Of Methyl And Hydroxypropyl Methylcellulose. Journal Of Applied Polymer Science 24 (1979); 1073–1087.
33. Mundada A., Satturwar P., Fulzele S., Joshi., Dorle A. Characterization And Evaluation Of Novel Film Forming Polymer For Drug Delivery. Iranian Journal Of Pharmaceutical Research 10(1) (2011); 35-42.
34. Machado R., Palmeira-De-Oliveira A., Martinez-De-Oliveira J., Palmeira-De-Oliveira R. Vaginal Films For Drug Delivery. Journal Of Pharmaceutical Sciences 102(7) (2013); 2069–2081.
35. Frey P. Film Strips And Pharmaceuticals. Pharmaceutical Manufacturer And Packing Sourcer. (2006); 92-93.
36. Wu X., Wang Y., Yang W., Xie B., Yang M., Dan W. A Rheological Study On Temperature Dependent Microstructural Changes Of Fumed Silica Gels In Dodecane. Soft Matter 8 (2012); 10457 – 10463.
37. Batista A., Nunes M., Fradinho P., Gouveia L., Sousa I., Raymundo A., Franco J. Novel Foods With Microalgal Ingredients – Effect Of Gel Setting Conditions On The Linear Viscoelasticity Of Spirulina And Haematococcus Gels. Journal Of Food Engineering 110 (2012); 182 – 189.
38. Bogatyrev V., Piskunov V., Shchegolev S., Ptichkina N. Fluctuation-Optical Method For Determining Gelation Point Of Biopolymer Systems. Colloid Journal 63(3) (2001); 270–274.
39. Cumper C., Alexander A. The Viscosity And Rigidity Of Gelatin In Concentrated Aqueous Systems. Australian Journal Of Chemistry 5(1) (1952); 146–152.
40. Medina-Esquivel R., Freile-Pelegrin Y., Quintana-Owen P., Yáñez-Limón J., Alvarado-Gil J. Measurement Of The Sol–Gel Transition Temperature In Agar. International Journal Of Thermophysics 29 (2008); 2036–2045.
41. Chung Y., Simmons K., Gutowska A., Jeong B. Sol-Gel Transition Temperature Of PLGA-G-PEG Aqueous Solutions. Biomacromolecules 3 (2002); 511-516.
42. Raghavan S., Cipriano B. Gel Formation: Phase Diagrams Using Tabletop Rheology And Calorimetry. Chapter 8 – Molecular Gels: Materials With Self-Assembled Fibrillar Networks – Springer The Netherlands (2005); 233 – 244.
43. Kanaya H., Hara K., Nakamura A., Hiramatsu N., Matsushige K. Investigations Of Pressure And Temperature Effects On Gelation Process Of Egg White By Time-Resolved Turbidimetric Measurements. Japanese Journal Of Applied Physics 33 (1994); 2817–2820.
44. Matsunaga T., Shibayama M. Gel Point Determination Of Gelatin Hydrogels By Dynamic Light Scattering And Rheological Measurements. Physical Review 76 – The American Physical Society. (2007); 0304011-0304014.
45. Sekiguchi Y., Sawatari C., Kondo T. A Gelation Mechanism Depending On Hydrogen Bond Formation In Regioselectively Substituted O-Methylcelluloses. Carbohydrate Polymers 53 (2003); 145–153.

46. Boudhani H., Laine C., Fulchiron R., Bounor-Legare V., Cassagnau P. Viscoelastic And Mechanical Properties Of Reactive PVC Plastisols. Polymer Engineering And Science 49(6) (2009); 1089–1098.
47. Barrera C., Florian-Algarin V., Acevedo A., Rinaldi C. Monitoring Gelation Using Magnetic Nanoparticles. Soft Matter 6 (2010); 3662–3668.
48. Wang Q., Xu H., Yang X., Yang Y. Rheological Study Of Aqueous Dispersions Of In Situ Gelable Thermosensitive Polymer Nanogels. Polymer Engineering And Science 49(1) (2009); 177–181.
49. Zhang Y., Xu X., Zhang L. Gel Formation And Low-Temperature Intramolecular Conformation Transition Of A Triple-Helical Polysaccharide Lentinan In Water. Biopolymers 89(10) (2008); 852–861.
50. Florian-Algarin V., Acevedo A. Rheology And Thermotropic Gelation Of Aqueous Sodium Alginate Solutions. Journal Of Pharmaceutical Innovation 5 (2010); 37–44.
51. Winter H., Chambon F. Analysis Of Linear Viscoelasticity Of Crosslinking Polymer At The Gel Point. Journal Of Rheology 30 (1986); 367 – 382.
52. Andueza I., Avila G., Attias D. Caracterización Física De Hidroxipropilmetilcelulosa Con Potencial Aplicación Oftalmológica: Ph, Tensión Superficial, Característica De La Película. Revista De La Sociedad Química De México 44(3) (2000); 224–228.
53. Thai L., Tomlinson A., Simmons P. In Vitro And In Vivo Effects Of A Lubricant In A Contact Lens Solution. Ophthalmic And Physiological Optics 22 (2002); 319–329.
54. Bhavsar A., Bhavsar S., Jain S. A Review On Recent Advances In Dry Eye: Pathogenesis And Management. Oman Journal Of Ophthalmology 4(2) (2011); 50–56.
55. Toda I., Shinozaki N., Tsubota K. Hydroxypropyl Methylcellulose For The Treatment Of Severe Dry Eye Associated With Sjogren's Syndrome. Cornea 15(2) (1996); 120–128.
56. Castellanos E., Iraizoz A., Bataille B., Brouillet F., Caraballo I. Estimation Of The Percolation Thresholds In Ternary Lobenzarit Disodium–Dextran–HPMC Hydrophilic Matrices Tablets: Effects Of Initial Porosity. European Journal Of Pharmaceutical Sciences 38 (2009); 312–319.
57. Fuertes I., Caraballo I., Miranda A., Millan M. Study Of Critical Points Of Drugs With Different Solubilities In Hydrophilic Matrices. International Journal Of Pharmaceutics 383 (2010); 138–146.
58. Veríssimo M., Pais A., Gomes M. Following HPMC Gelation With A Piezoelectric Quartz Crystal. Carbohydrate Polymers 82 (2010); 363–369.
59. Hu G., Li H., Wang X., Zhang Y. Measurement And Correlation Of Griseofulvin Solubility In Different Solvents At Temperatures From (281.95 To 357.60) K. Journal Of Chemical Engineering Data 55 (2010); 3969–3971.

60. Gariepy, E., Leroux, J. In Situ-Forming Hydrogels – Review Of Temperature-Sensitive Systems. European Journal Of Pharmaceutics And Biopharmaceutics 58(2004); 409–426.
61. Suzuki K., Yoshihiro T., Enomoto T. The Effect Of Pressure On The Sol-Gel Transformations Of Macromolecules. Bulletin Of The Chemical Society Of Japan 45 (1972); 336–338.
62. Haque A., Morris E. Thermogelation Of Methylcellulose. Part I: Molecular Structures And Processes. Carbohydrate Polymers 22 (1993); 161 – 173.
63. Liu S., Joshi S., Lam Y., Tam K. Thermoreversible Gelation Of Hydroxypropyl Methylcellulose In Simulated Body Fluids. Carbohydrate Polymers 72 (2008); 133–143.

2. EFFECT OF THE ADDITION OF FLAVORANTS ON THE GELATION TEMPERATURE OF HPMC SOLUTIONS

2.1 Introduction

Biopolymer films as drug delivery method have significant advantages when compared with traditional hydrophobic drugs formulations. For these applications thermoreversible polymer matrices are proposed, since these materials have the ability to form gels and encapsulate the drug particles in the intermolecular spaces of the network providing high stability over time.[1-3]

Many edible gel systems have been proposed as food coating and drug delivery methods. [4,5] Most of these systems have common formulation components, such as a biopolymer matrix, plasticizers, surfactants, an active ingredient (drug, nutraceutical, essential oil, etc), flavorants, and in some cases artificial coloring.[6]

Several methods for the preparation of these thin films have been proposed. Casting [7], extrusion [8], spraying [9], and knife-coating [10] are some of them. Casting method is extensively used in the preparation of films in laboratory scale where drying conditions can vary from 12 min with hot air to 12 hours at room temperature, while the continuous casting method is mainly used in industrial scale.[11]

The casting method basically consists of spreading a polymer-solvent-additives precursor solution onto a flat non-stick surface, evaporate the solvents on the solution controlling the environment around the sample, from room temperature convection, to the use of vacuum/convection ovens, or hot plates, and finally removing the formed film. The most common solvents used in films preparation by casting method are water and ethanol.[12]

As stated before, HPMC gelation mechanism is led by hydrophobic interactions in its final stage, which occur due to the disturbance of the water cages structures around the hydrophobic groups in the polymer chain promoting the interactions between polymer chains. In this process, factors such as the molecular weight of the polymer, its degree of substitution (mainly for the methoxy groups), and the quality of the solvent have a significant effect.

Regarding the quality of the solvent, it has been reported that the incorporation of ethanol and propylene glycol has an inhibitor effect on the gelation process for HPMC solutions, as for other small molecules such as glycerol, sorbitol, and some salts lower the gel point. These behaviors are related to the effect of the addition of these compounds in the dehydration of the polymer chains. In this chapter the evaluation of the effect of the addition of small, soluble or partially soluble molecules, to the solvent mixture is proposed, to understand how the solubility of the polymer and the quality of the solvent affect the gelation mechanism.

Due the low water solubility of some flavorants, the use of a solvent mixture 25% ethanol and 75% water was used to enhance the solubility of these compounds; despite this, concentrations below 0.1% were used because of solubility restrictions. Flavorants were chosen because are small, partially soluble molecules capable of interact with both the aqueous and organic part of the solvent mixture and could be used as models to predict the effect of the addition of compounds with similar chemistry. These compounds exhibit different chemical functionalities depending of their main functional group. In this study, alcohols, aldehydes, esters, and monoterpenes flavorants were chosen.

Molecular interactions between the polymer and the complex solvent mixture (water:ethanol:flavorant) may be beneficial or detrimental for the gelation process, and these interactions must be previously known and understood within a film formulation process.

2.2 Experimental section

2.2.1 Materials

As states before, in this study a polymer matrix and different flavorants organized by functional group were evaluated. Hydroxypropyl methylcellulose (HPMC) grade E4M (CAS-No: 9004-65-3) was purchased from Sigma-Aldrich.

Alcohol flavorants: eugenol (CAS-No: 97-53-0), cinnamyl alcohol (CAS-No: 104-54-1), citronellol (CAS-No: 106-22-9).

Ester flavorants: cinnamaldehyde (CAS-No: 104-55-2), ethyl vanillin (CAS-No: 121-32-4), vanillin (CAS-No: 121-33-5).

Aldehyde flavorants: ethyl cinnamate (CAS-No: 103-36-6), menthyl acetate (CAS-No: 89-48-5), terpinyl acetate (CAS-No: 80-26-2).

Monoterpene flavorants: eucalyptol (CAS-No: 470-82-6), geraniol (CAS-No: 106-24-1), menthol (CAS-No: 89-78-1), were all purchased from Sigma-Aldrich and used without any modification. Ethanol (CAS-No: 64-17-5), and deionized water were used as solvent for all samples.

2.2.2 Sample Preparation & Characterization

HPMC 1% (by weight) solutions were prepared using a mixture of deionized water-ethanol as solvent. Ethanol as part of the solvent mixture was needed to improve the solubility of the flavorants compounds. Previous measurements were made to determine the best ethanol:water ratio, in order to enhance the solubility of the flavorants without significant effect on the gelation temperature of the solution. A value of 1:3 ethanol:water was chosen for all samples.

The solvent mixture was heated above 80 °C. The polymer was added gradually with continuous stirring to avoid agglomeration and enhance its solubility. Flavorants were added after dissolution of the polymer in the solvent mixture. Concentrations of flavorants of 0.005, 0.01, 0.025, 0.05 and 0.1% were incorporated into the polymer solution. The solutions were then cooled and slowly stirred for 24 to 36 hours at room temperature. The final HPMC-flavorants solutions were used for the rheological measurements within the next seven days after preparation to avoid any contamination.

Rheological measurements were used to determine the gelation temperature of the samples. An ATS Rheologica Stresstech HR (stress-controlled) rheometer equipped with an ETS temperature controller; a double-gap Couette fixture was used for these experiments. All samples were left to rest for twenty minutes after loading to equilibrate stresses and temperature. A heating ramp from 25 to 80 °C at a heating rate of 0.5 °C/min, was then applied during an oscillatory shear experiment, at a constant strain of 0.01 and frequency of 1 Hz, from which the loss (G'') and storage (G') moduli were obtained as a function of temperature. The gel point was extracted from the data, as the point where $G'= G''$. All measurements were performed in triplicate and results correspond to the average ± the standard deviation. A two-way ANOVA (analysis of variance) was performed for all the data collected to evaluate the effect of the type of functional group (alcohol, ester, aldehyde, monoterpene) present in the flavorants and its concentration on the gelation temperature of the HPMC solutions.

Before rheological characterizations, flavorants were organized by their main functional group, in order to evaluate the effect of the interaction of these groups with the polymer and how this affect the gelation process (T_{gel}). Four main functional groups were categorized: alcohols, esters, aldehydes and monoterpenes. A factorial experimental design as seen on the Table 1 was developed to evaluate both the effect of the functional groups and the effect of the concentration of flavorant on T_{gel} by rheological measurements. Results are presented by functional group to evaluate first the effect of the concentration of the different flavorants on T_{gel}, and then a global ANOVA will be shown to evaluate the effect of the functional groups as a whole.

Table 1. Factorial experimental design to determine the effect of flavorant functional group and concentration on Tgel of a HPMC in a water/ethanol mixture

Functional group	Molecule	Concentration (weight %)
alcohol	cinnamyl alcohol	0, 0.005, 0.01, 0.025, 0.05, 0.1
	eugenol	0, 0.005, 0.01, 0.025, 0.05, 0.1
	citronellol	0, 0.005, 0.01, 0.025, 0.05, 0.1
ester	ethyl cinnamate	0, 0.005, 0.01, 0.025, 0.05, 0.1
	menthyl acetate	0, 0.005, 0.01, 0.025, 0.05, 0.1
	terpinyl acetate	0, 0.005, 0.01, 0.025, 0.05, 0.1
aldehyde	cinnamaldehyde	0, 0.005, 0.01, 0.025, 0.05, 0.1
	ethyl vanillin	0, 0.005, 0.01, 0.025, 0.05, 0.1
	vanillin	0, 0.005, 0.01, 0.025, 0.05, 0.1
monoterpene	eucalyptol	0, 0.005, 0.01, 0.025, 0.05, 0.1
	geraniol	0, 0.005, 0.01, 0.025, 0.05, 0.1
	menthol	0, 0.005, 0.01, 0.025, 0.05, 0.1

ANOVA analyses are statistical method based on linear regression mathematical models, which may determine whether different treatments or conditions exhibited significant

differences compared with a tolerance level, or otherwise can be assumed that their population means do not differ. For the use of ANOVAs in data analysis, three presuppositions or hypothesis must be true: Independence in the observations, residuals distribution must be normal, and must be equal variances between the samples. For the latest, Bartlett's [13] (parametric) test is used to evaluate the variance distribution assuming normal distribution of the data, and Levene's [14] tests was used when non-parametric distribution (non-normal distribution) is expected, p-values above 0.05 for both test indicate an equal variance for all measurements. This method will be discussed in detail in the appendix for this chapter.

In statistics, a significance level is established to accept or reject the null hypothesis (hypothesis that propose that all the results are equal). For a 95% of confidence a p-value of 0.05 is fixed. If the p-values of the different evaluated factors evaluated are below 0.05 the null hypothesis is rejected, and then we can assure that the measurements are different. In this work, two factors were evaluated. The first was the flavorant and has three levels, for alcohols the levels are 1-citronellol, 2-eugenol, 3-cinnamyl alcohol, while the second factor have six levels and represents all the concentrations tested (0, 0.005, 0.01, 0.025, 0.05 y 0.1 weight %). In addition the interaction of these two factors also is evaluated.

2.3 Results and discussion

2.3.1 Alcohol flavorants

Three alcohol flavorants were chosen for these section, citronellol, eugenol and cinnamyl alcohol, which are small molecules with similar structure, a cyclic hydrocarbon (or semi-cyclic), and hydroxyl (–OH) groups (Figure 6). The hydroxyl groups in the alcohols may interact with the water portion of the solvent reducing the amount of hydrogen-hydrogen interactions between the solvent and the polymer, and this in turn decrease the solubility of the polymer reducing the hydrophilic interactions between polymer chains responsible of the gelation, [15] so that one can anticipate an increase in the gelation temperature (T_{gel}).

Figures 7, 8, and 9, illustrate the behavior of the T_{gel} for all three alcohol flavorants over the entire concentration range. With the addition of low concentrations of alcohol flavorants, an initial decrease in T_{gel} is observed, which is attributed to an initial saturation of the solution with the flavorant. Afterwards that initial saturation, an inhibition in the gelation of HPMC can be observed as higher concentrations are added. The latter is attributed to the increase in the amount of aromatic hydrocarbon groups in the solvent, which decreases the solubility of the polymer by solubilizing the polymer molecules in the solvent mixture, a main factor in the HPMC gelation mechanism.[16]

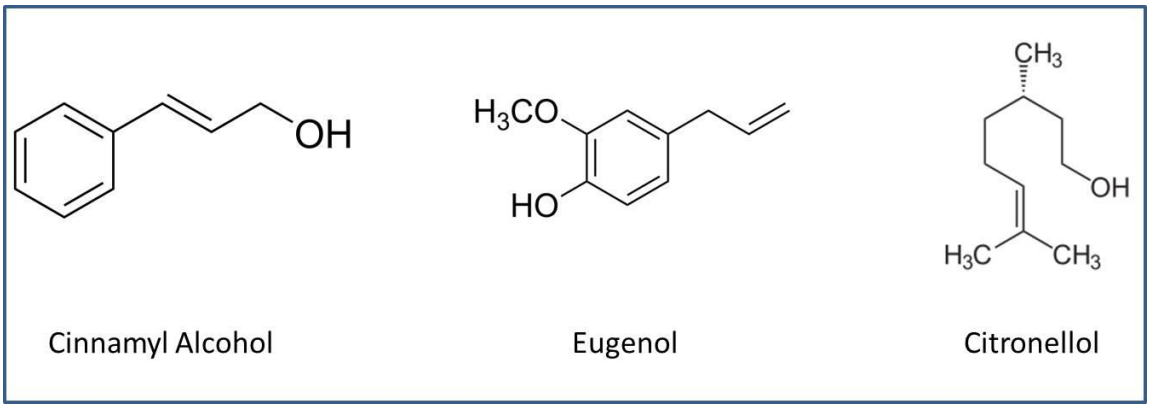


Figure 6. Cinnamyl alcohol, eugenol and citronellol molecular structure

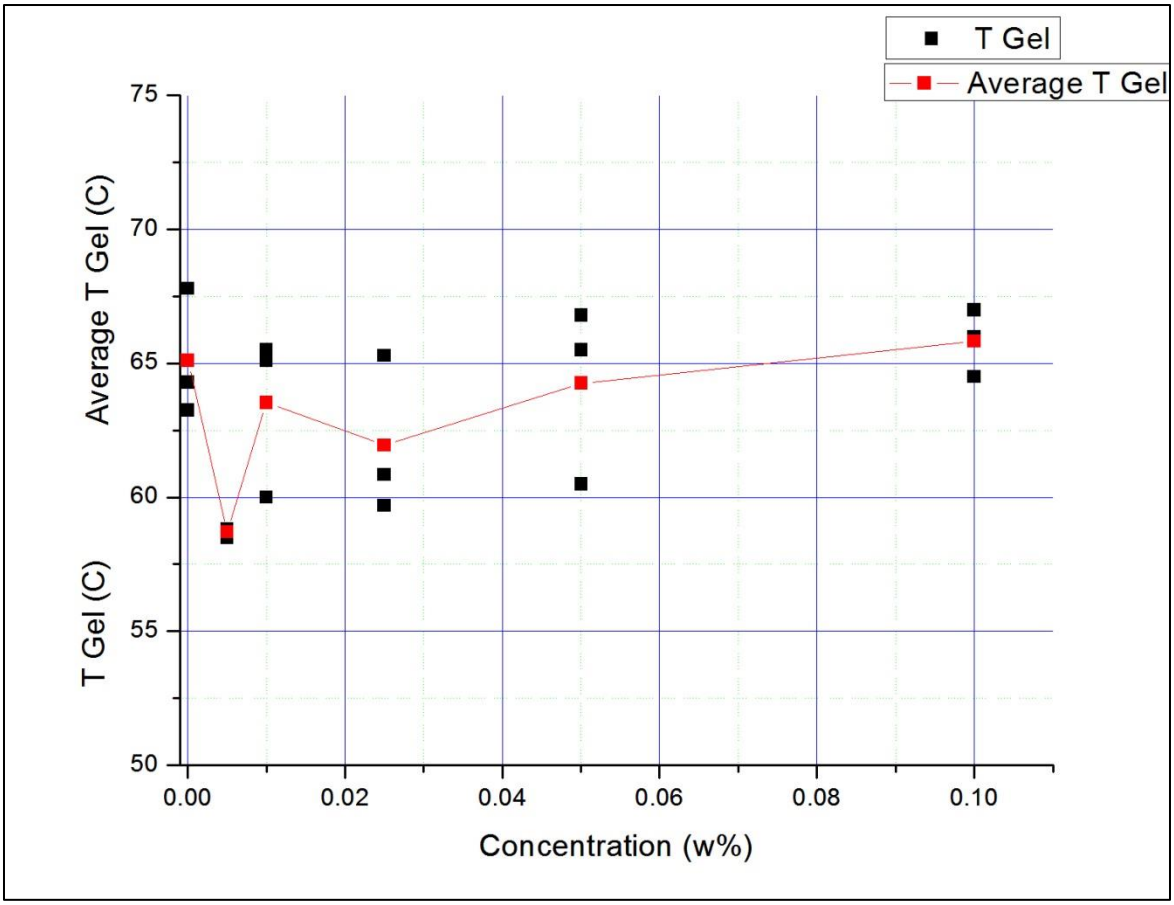


Figure 7. Effect of the concentration of cinnamyl alcohol on T_{gel} of HPMC solutions with a water:ethanol mixture as solvent

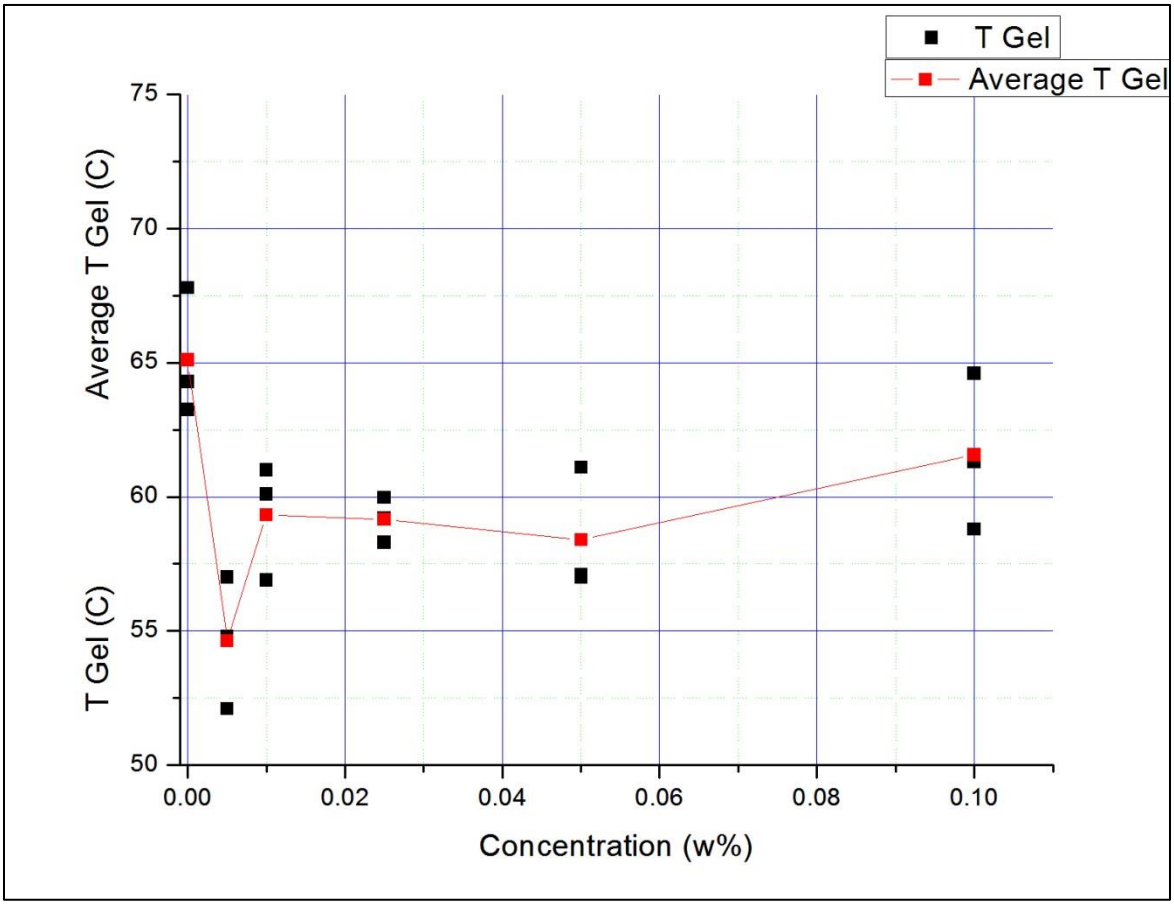


Figure 8. Effect of the concentration of eugenol on T_{gel} of HPMC solutions with a water:ethanol mixture as solvent

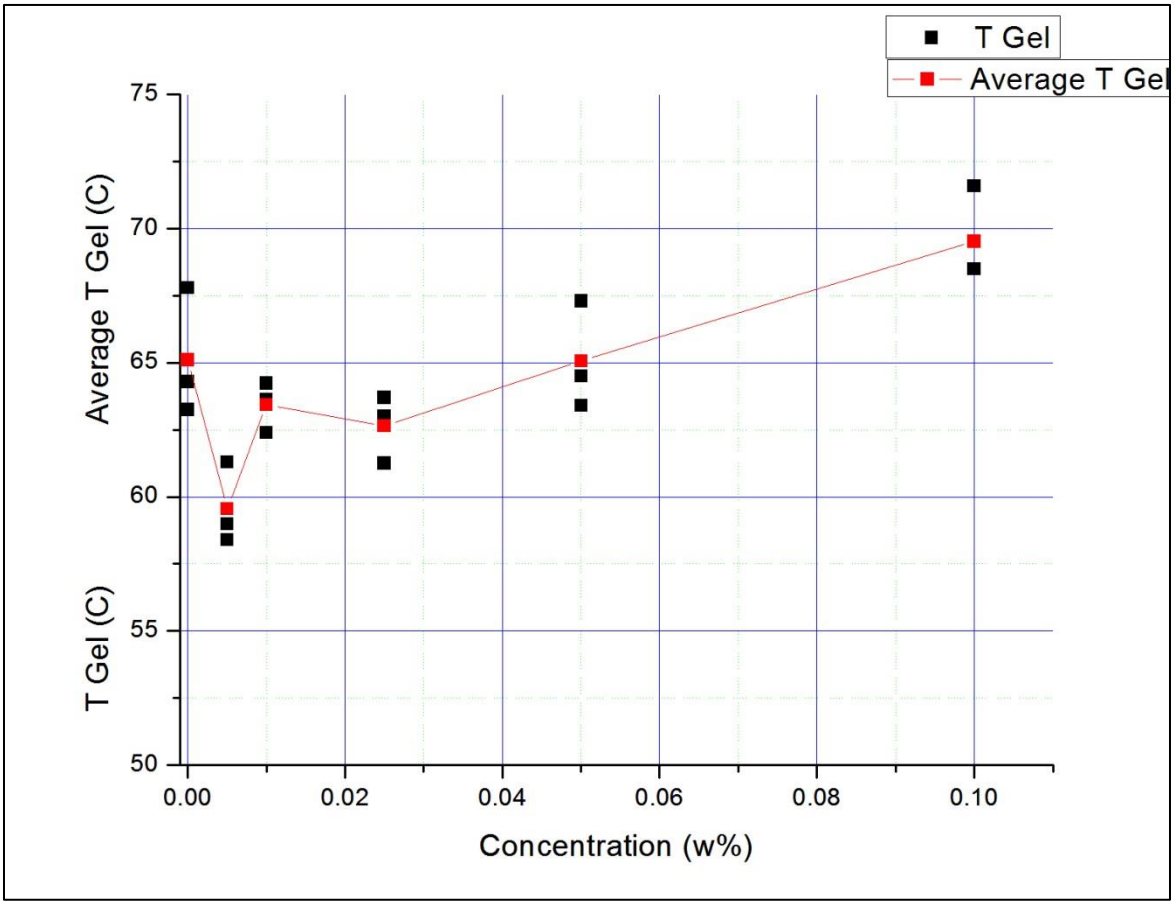


Figure 9. Effect of the concentration of citronellol on T_{gel} of HPMC solutions with a water:ethanol mixture as solvent

A trend of increasing the gelation temperature whit increasing the concentration of the three different alcohol flavorants was observed. From the ANOVA results, p-values for type of flavorant and concentration are below the significance level (0.05), so it can be concluded that both factors have a significant effect on the gelation temperature (more details of the calculations on the appendix for this chapter).

The main effect of the presence of flavorants in the solvent mixture on the gelation temperature of the HPMC solutions can be attributed to changes in the solubility of the polymer in the solvent due to the solubilizing effect that alcohol molecules have on the polymer molecules. With the additions of these flavorant molecules to the solvent, interactions between the hydrophobic sites of the polymer are reduced, and the energy required for disturb the water cages structures surrounding the hydrophobic groups in the polymer chains increases, so is the gelation temperature.

The gelation mechanism for HPMC is based on two main stages of structures formation.[17] Changes in the solvent composition caused by the addition of small partial soluble molecules as flavorants may affect this process, reducing the freedom of movement of the polymer chains in solution.

Hansen solubility parameter calculations were made to visualize the effect of the addition of flavorants to the quality of the solvent in the gelation process of the polymer solutions, and will be discussed in detail on the section 2.3.5.

2.3.2 Ester flavorants

Esters flavorant used in this work are fairly soluble in water. Although esters are not able to form hydrogen bonds with each other they can with water molecules. However, its solubility decays with increasing chain length. In this work small ester molecule flavorants

as ethyl cinnamate, menthyl acetate and terpinyl acetate were used (Figure 10) to ensure better dispersion in the solvent.

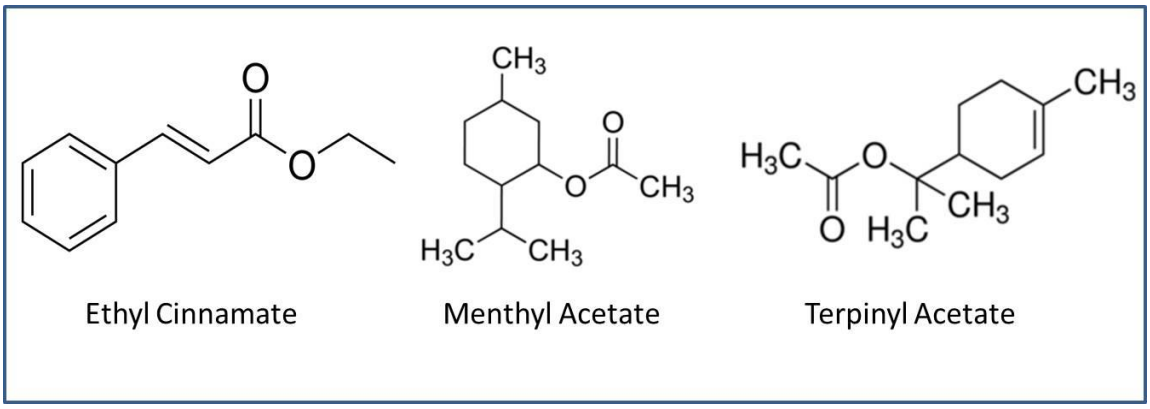


Figure 10. Ethyl cinnamate, menthyl acetate and terpinyl acetate molecular structure

To dissolve a small ester molecule, one of the partially-positive hydrogen atoms in a water molecule is attracted to one of the lone pairs on one of the oxygen atoms in the ester forming a hydrogen bond. Dispersion forces and dipole-dipole attractions are also present in the process; however the solubility of organic part of the molecule decreases with increasing the temperature.[18] The presence of these intermolecular interactions lowers the energy needed to dissolve the ester molecule. As the molecule chain length increases, the organic portion of the esters gets between water molecules breaking the fairly strong hydrogen bonds between water molecules without offering an energetic compensation.

Additionally, the water molecules are forced into an ordered alignment along the chain, reducing the entropy in the system. This makes the process thermodynamically less favorable, and so solubility decreases.[19]

Figures 11, 12, and 13, show the results for T_{gel} of HPMC solutions when esters flavorants are added in the studied range of concentration. The gelation temperature of the HPMC solutions in the presence of ester-flavorants remains almost constant since there is no significant variation in the studied range.

From the ANOVA results, p-values for flavorant and concentration are above the significance level (0.858 and 0.070 respectively) supporting the conclusions from the rheological observations (more details of the calculations are discussed on the appendix for this chapter).

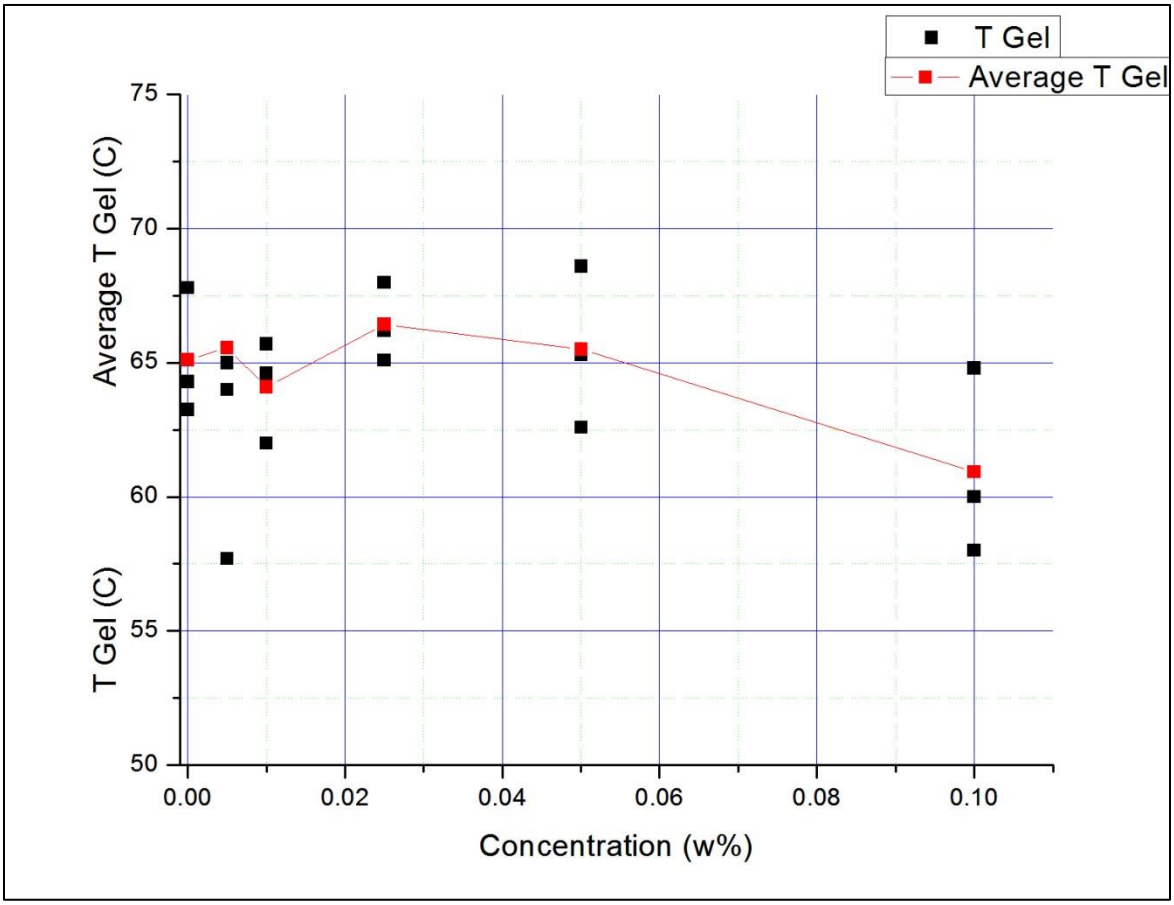


Figure 11. Effect of the concentration of ethyl cinnamate on T_{gel} of HPMC solutions with a water:ethanol mixture as solvent

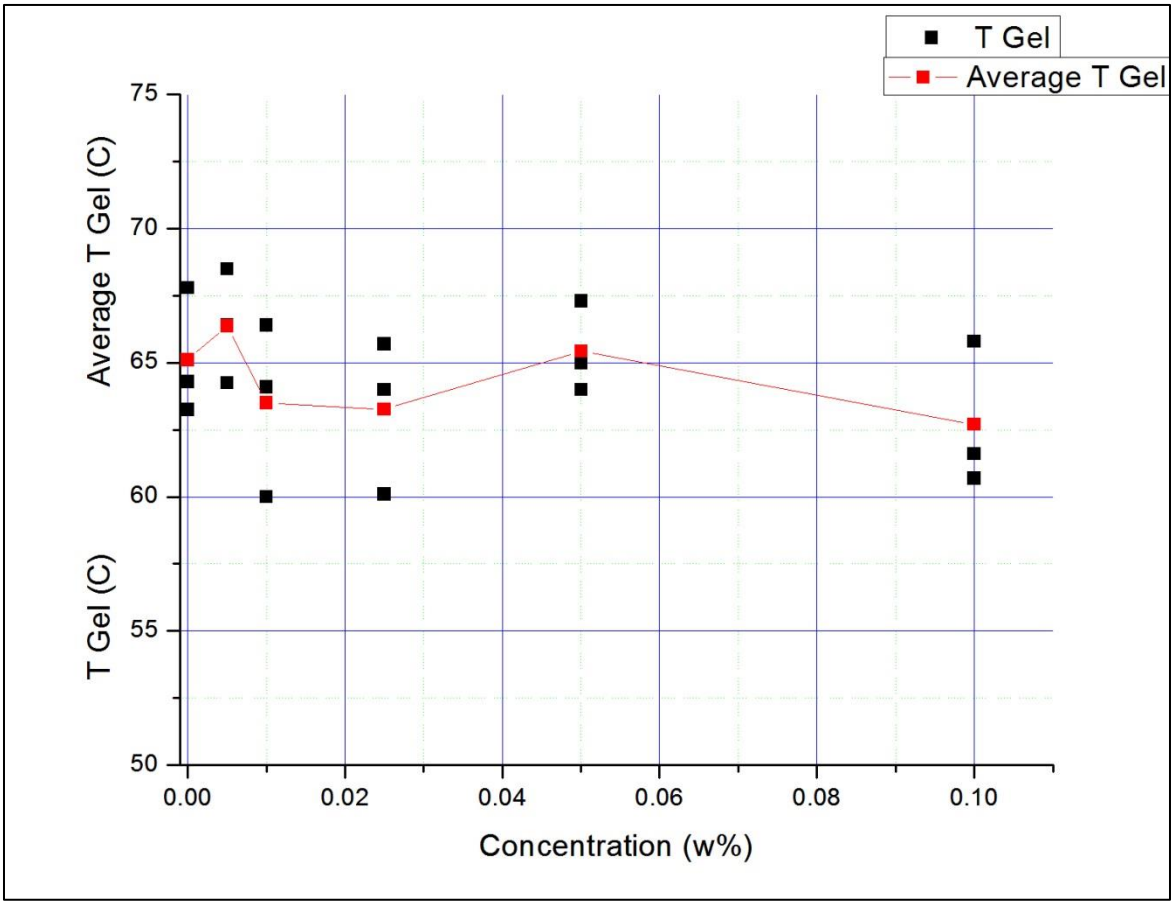


Figure 12. Effect of the concentration of menthyl acetate on T_{gel} of HPMC solutions with a water:ethanol mixture as solvent

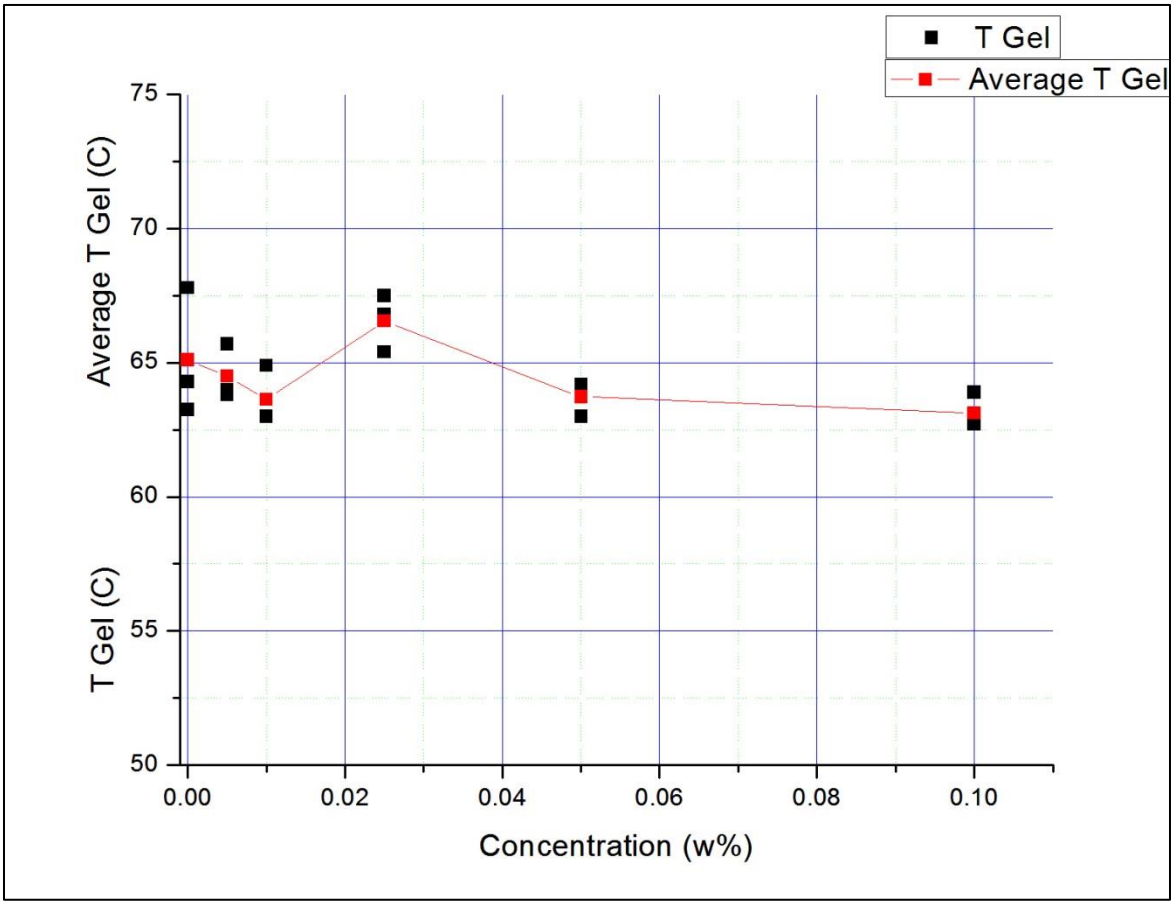


Figure 13. Effect of the concentration of terpinyl acetate on T_{gel} of HPMC solutions with a water:ethanol mixture as solvent

2.3.3 Aldehyde flavorants

The aldehyde flavorants used in this work are slightly soluble in water, but highly soluble in organic solvents like ethanol. Hydroxyl groups present in the flavorants interact with water molecules by H-H bonds. Nevertheless, the organic part of the molecule interacts mostly with the organic part of the solvent (ethanol).

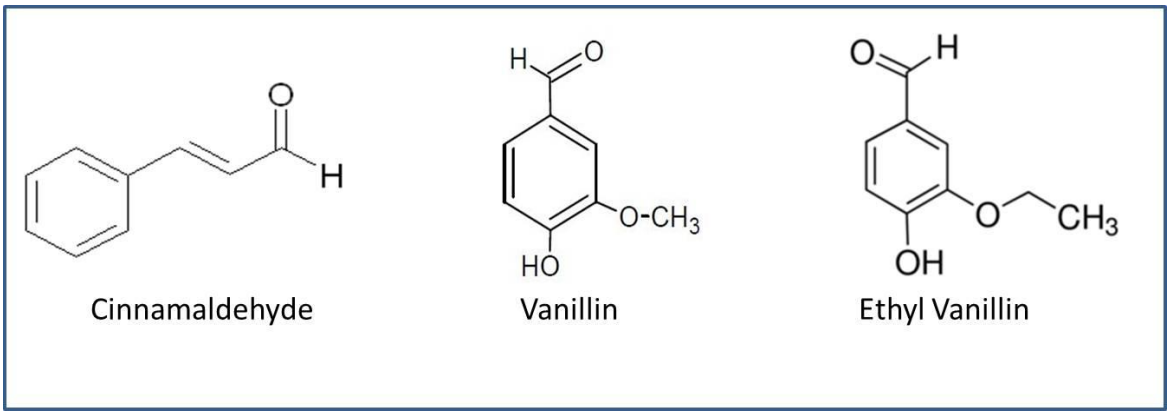


Figure 14. Cinnamaldehyde, vanillin and ethyl vanillin molecular structure

Similar to esters, aldehyde molecules are slightly soluble in water, and its solubility decrease with increasing the chain length. The solvation mechanism of aldehydes is very similar to esters molecules. A lone pair of electrons on the oxygen in the carbonyl group (-C=O) forms a hydrogen bond with a slightly positive hydrogen on the water molecule. As the chain length increase, the hydrocarbon part of the aldehyde molecule starts to inhibit the H-H bonds between water molecules making the process energetically less efficient and decreasing the solubility of the polymer in the solvent. This process may interfere with the water cages dehydration around the hydrophobic substituents in the polymer chain, delaying the gel structure formation.

From the rheological characterization (Figures 15, 16, 17), an increase in the gelation temperature of the samples can be observed at higher concentrations of vanillin and ethyl vanillin. On the other hand, cinnamaldehyde causes a slight decrease which is later proved to be statistically insignificant. All the data was previously tested to ensure statistical

consistency (the results for the equal variance test, and ANOVA calculations are presented on the appendix).

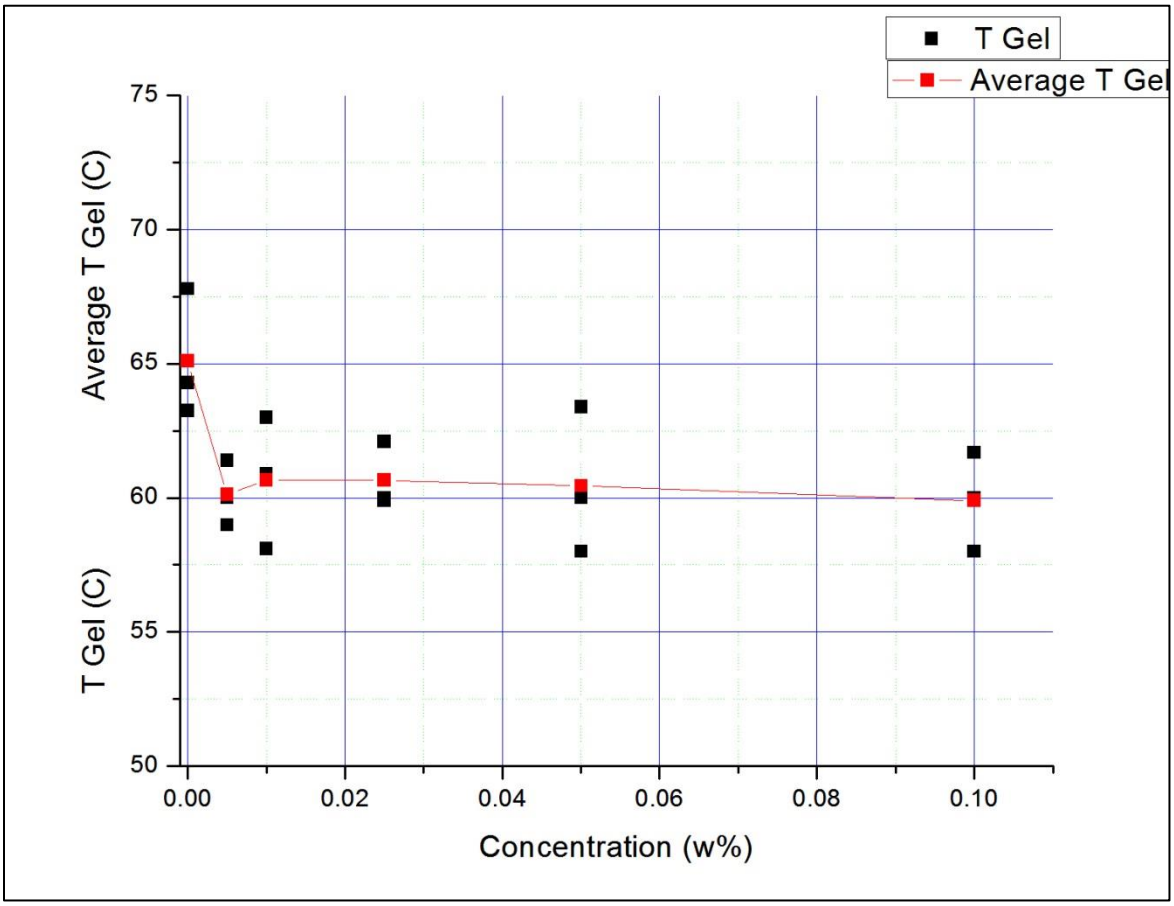


Figure 15. Effect of the concentration of cinnamaldehyde on T_{gel} of HPMC solutions with a water:ethanol mixture as solvent

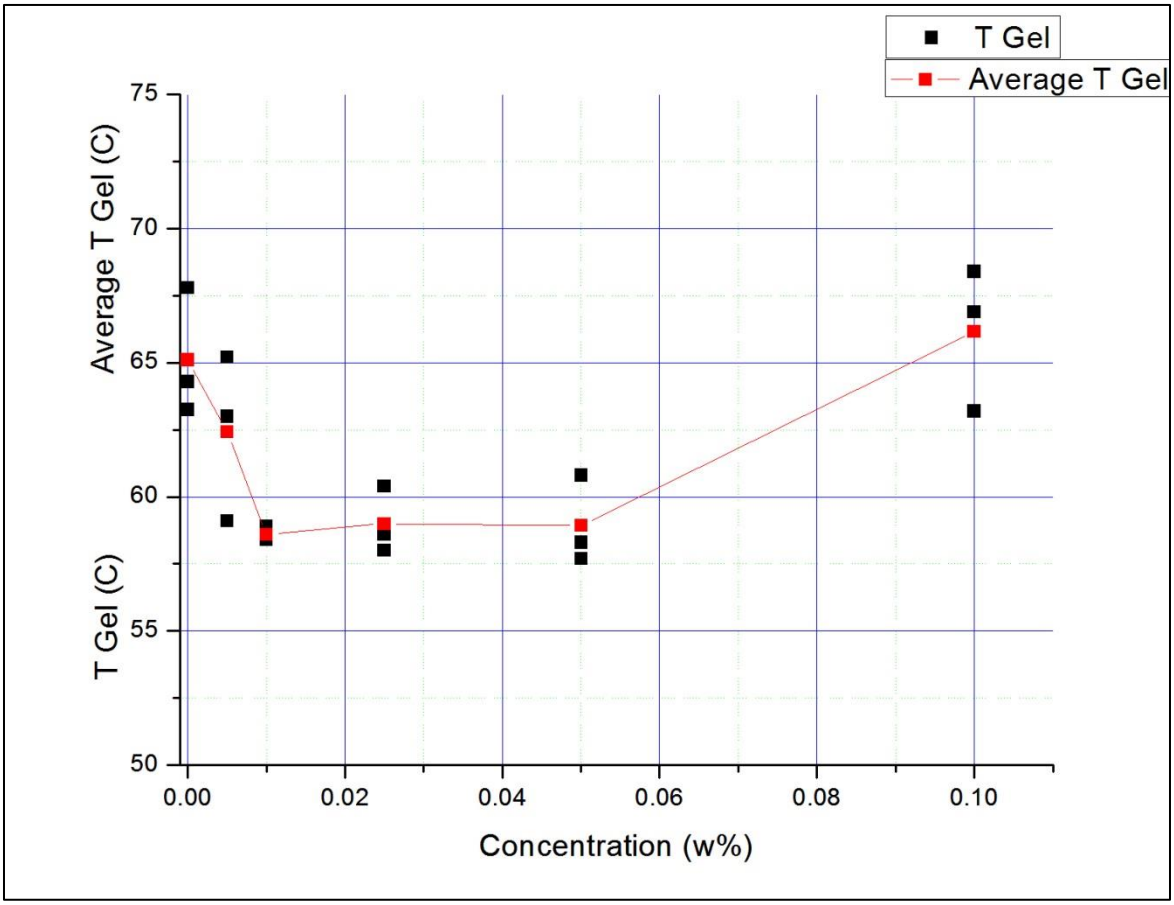


Figure 16. Effect of the concentration of vanillin on T_{gel} of HPMC solutions with a water:ethanol mixture as solvent

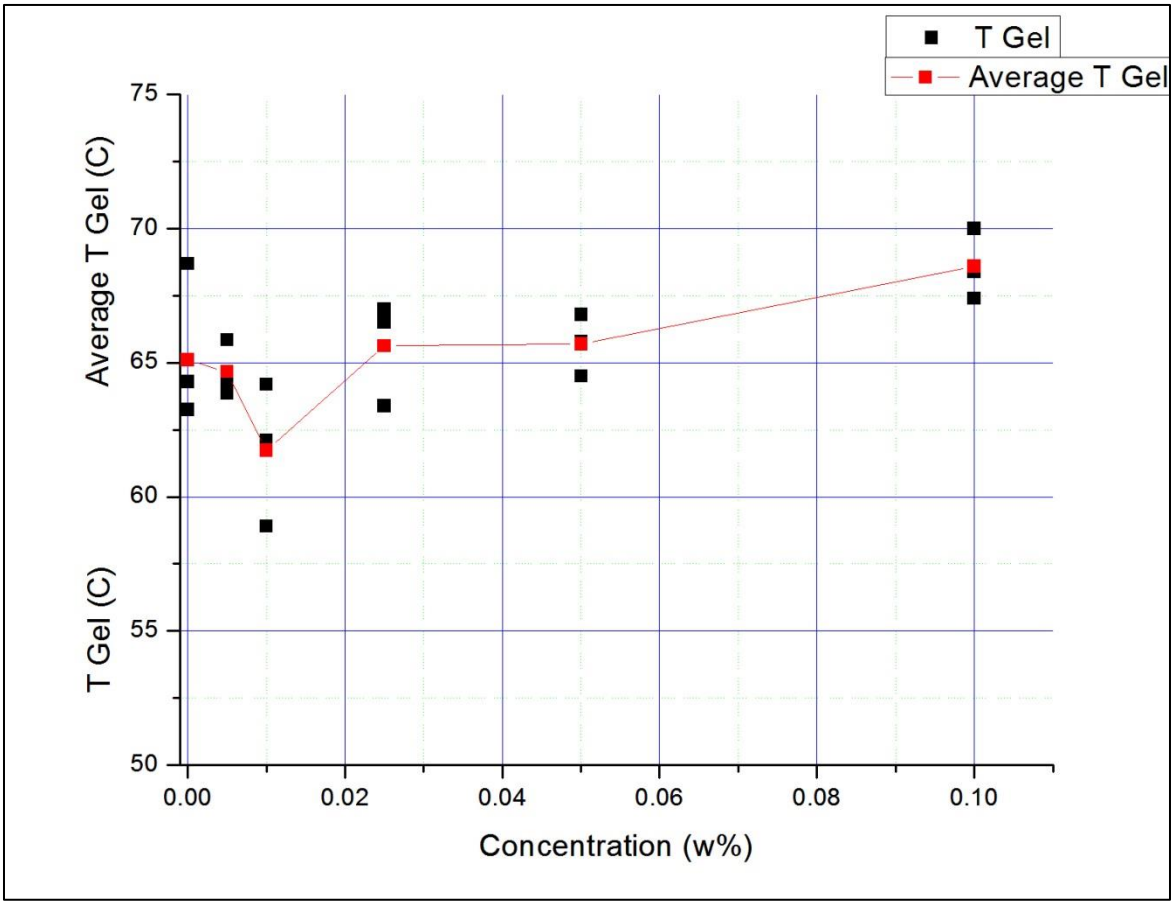


Figure 17. Effect of the concentration of ethyl vanillin on T_{gel} of HPMC solutions with a water:ethanol mixture as solvent

The main difference observed from the rheological data is that cinnamaldehyde does not have a significant effect on the gelation process, which is attributed to the lower number of hydroxyl groups per molecule reducing the ability to solubilize the polymer reducing the polymer-polymer hydrophobic interactions responsible of the gel formation. Since vanillin and ethyl vanillin have more hydroxyl groups in their structure, they are expected to be able to solubilize the polymer and reduce the hydrophilic interactions between the

polymer chains, increasing the energy barrier necessary for the gelation process too and, therefore, increasing T_{gel}.

The conclusions from the rheological measurements were supported with the ANOVA results, which shown that the three factors evaluated (flavorant, concentration and the interactions) have a significant effect on the gelation temperature of the sample, with p-values of 0.000, 0.000 and 0.003 respectively. Values of p-values below three significant figures are reported as 0.000 by Minitab.

In this case, the difference in the amount of hydroxyl groups of the flavorant molecules and flavorant concentration, are responsible for the changes during the gelation process. A synergistic interaction of factors has also a significant effect on the gelation temperature of the HPMC solutions.

2.3.4 Monoterpene flavorants

Monoterpenes are small acyclic, monocyclic, bicyclic or tricyclic molecules.[20] They are also non-polar, and highly volatile compounds. Some of them have been reported as antibacterial compounds.[21] The monoterpene flavorants used in this work (Figure 18) are lightly soluble in water (in the range of 0.1 – 3.5g/L) but highly soluble in lipids. In overall, a generalization has been made that monoterpene compounds are insoluble in water.[22, 23] However, monoterpenes containing oxygen in the form of an alcohol,

ketone, aldehyde or ester have solubilities 10-100 times greater than hydrocarbons with comparable skeletons.[24]

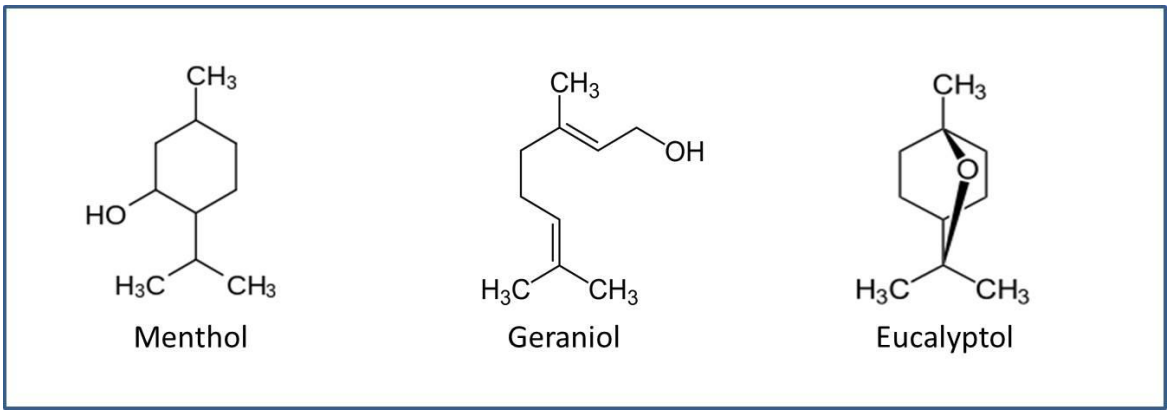


Figure 18. Menthol, geraniol and eucalyptol molecular structure

Figure 19 and 20 illustrate the behavior of T_{gel} when menthol and geraniol are added in the studied concentration range to the HPMC solutions. These two compounds are alcohol-like monoterpene flavorants, and similar to their counterpart alcohol flavorants inhibit the gelation of HPMC, increasing the gelation temperature of the solutions. Eucalyptol (1,8-Cineole) on the other hand, does not have a significant effect on the gelation temperature of the HPMC solutions (Figure 21).

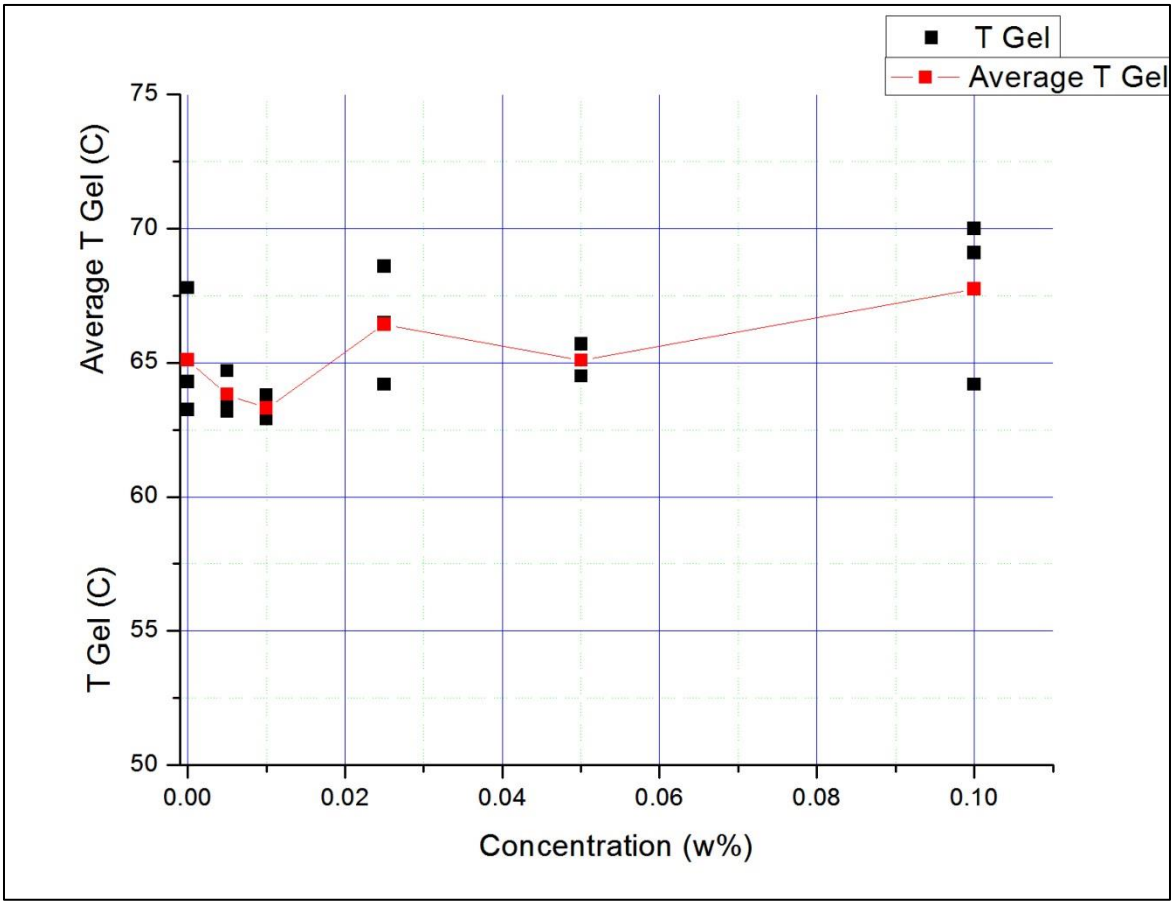


Figure 19. Effect of the concentration of menthol on T_{gel} of HPMC solutions with a water:ethanol mixture as solvent

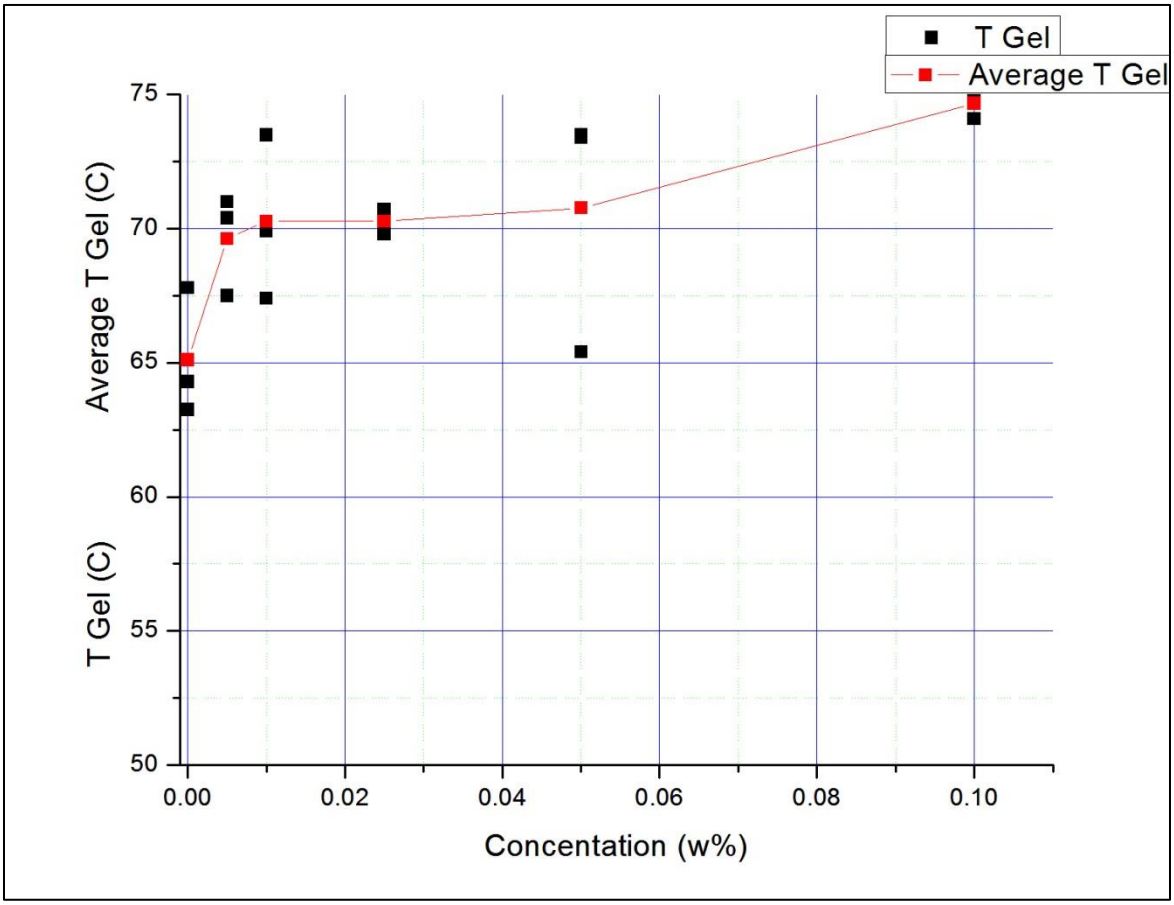


Figure 20. Effect of the concentration of geraniol on T_{gel} of HPMC solutions with a water:ethanol mixture as solvent

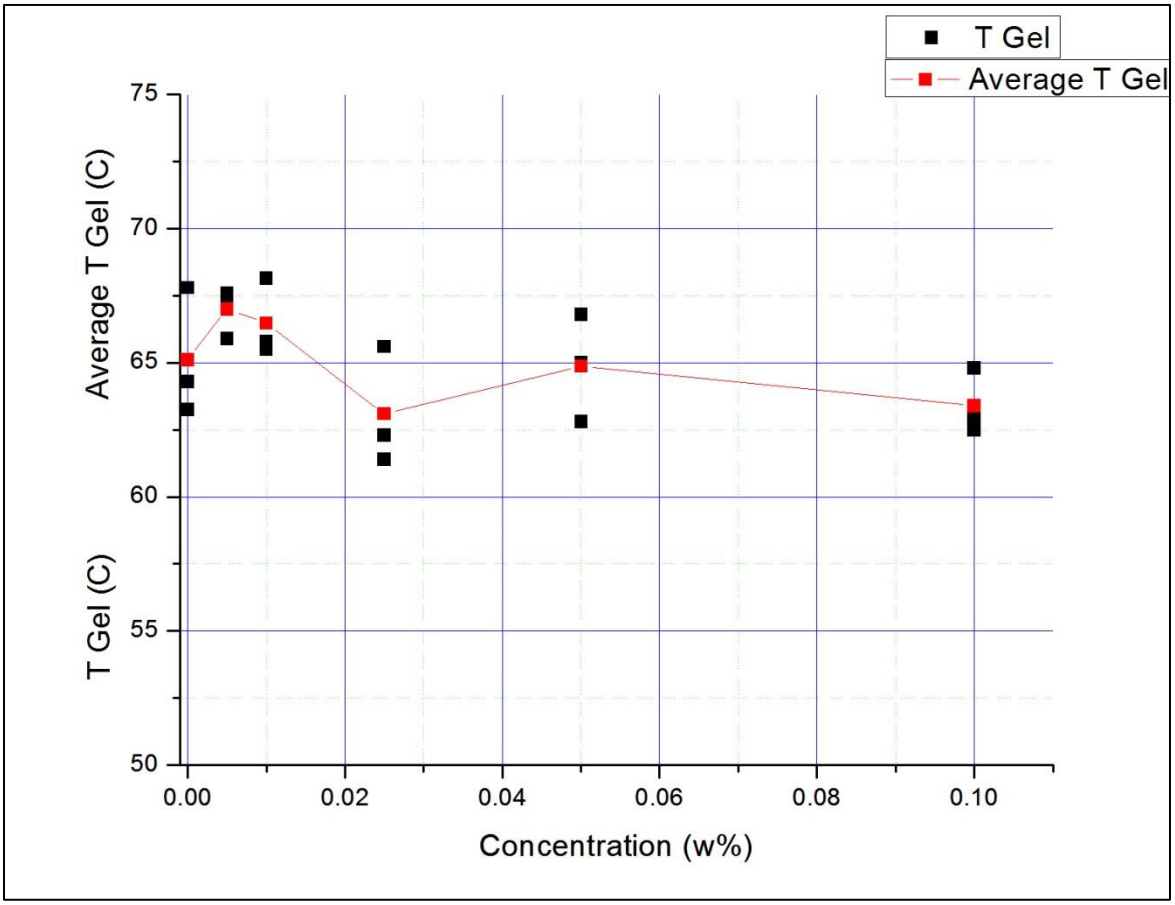


Figure 21. Effect of the concentration of eucalyptol on T_{gel} of HPMC solutions with a water:ethanol mixture as solvent

From the statistical analysis, it can be concluded that there is a slight effect corresponding to the concentration, and a significant effect of the type of flavorant, and the interaction of factors on the gelation temperature of the HPMC solutions, with p-values of 0.051, 0.000 and 0.002 respectively. P-values above 0.05 were found in both data validation tests, for parametric and non-parametric distributions which confirm that the data is statistically consistent (more detains in the appendix). A second ANOVA was performed only with menthol and geraniol, since these flavorants exhibited an alcohol-like behavior in the

rheological measurements, and the results shown that both flavorants have a significant effect on T_{gel} on the studied concentration range.

It can be concluded that the effect on the gelation of HPMC is directly related with the relative solubility of the flavorants in the two solvents (water-ethanol). The effect of the dispersion, dipolar intermolecular forces, and hydrogen bonds between the polymer and the solvent can be evaluated by calculating the Hansen solubility parameters [25, 26] for each possible solvent interaction with the polymer in the solution to understand deeply how the addition of the flavorants affect the gelation process of the HPMC solutions. -

2.3.5 Solubility parameters

As stated previously, solubility of the polymer chain in the solvent can be assumed as one of the main factors affecting the gelation mechanism of HPMC.[17, 27] The different molecular interactions between polymer and solvent are typically responsible for the dehydration of the water cages around the hydrophobic sites on the polymer chain, and thus the stretching of the molecule. Other factors that affect the gelation of HPMC are pH, substitution degree (amount of $–CH_3$ groups), and temperature.[28]

Solubility parameters were calculated to understand how the addition of flavorant to the solvent mixture affects the gelation process of HPMC. Hansen solubility parameters were calculated using the Hoftyzer-van Krevelen group-contribution method.[29] This method

was chosen because it allows for the calculation of the effects of the dispersion, dipole and hydrogen-bond forces, on the solubility of the polymer. The following set of equations compromise the method to calculate the solubility parameter (δ)

$$\delta_d = \frac{\sum F_{di}}{V} \qquad \text{(Eq. 1)}$$

$$\delta_p = \frac{\sqrt{\sum F_{pi}^2}}{V} \qquad \text{(Eq. 2)}$$

$$\delta_h = \sqrt{\frac{\sum E_{hi}}{V}} \qquad \text{(Eq. 3)}$$

$$\delta^2 = \delta_d^2 + \delta_p^2 + \delta_h^2 \qquad \text{(Eq. 4)}$$

Where the *d*, *p* and *h* subscript stand for dispersion, polar and hydrogen bond interaction contributions. The subscript *i* correspond to the number of functional groups present in the molecule. V is defined as the molar volume of the solvent, $V = \sum \phi_i V_i$, which is calculated using the volume (or molar) fraction (ϕ) of each component in the solvent mixture and their respective molar volumes, in this equation the subscript i correspond the number of compounds in the solvent mixture. In this work, the solvent was defined as the mixture between water-ethanol-flavorant, and the polymer (HPMC) as the solute.

The group contribution method, allows calculating the individual contribution of each structural group present in the flavorant molecule, through the *F* and *E* parameters (*F* represent the molar attraction components and *E* stands for the cohesive energy) as shown

on Table 2. For example, the contribution of small linear hydrocarbons has a strong contribution on the dispersion forces while cyclic carbon groups have stronger contributions in dispersion and polar interactions.

Table 2. Solubility parameter: component group contribution. Reproduced from Polymer Handbook 4th Edition volume 2. [30]

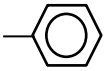
Structural group	F_{di} ($J^{1/2}$ $cm^{3/2}$/mol)	F_{pi} ($J^{1/2}cm^{3/2}$/mol)	E_{hi}(J/mol)
	1430	110	0
-CH3	420	0	0
>CH2	270	0	0
>CH-	80	0	0
>C<	-70	0	0
$=CH_2$	400	0	0
=CH-	200	0	0
=C<	70	0	0
-OH	210	500	20000
-O-	100	400	3000
-COH	470	800	4500
-CO-	290	770	2000

Table 3 summarizes the solubility parameters calculated by the Hoftyzer-van Krevelen method for all the flavorants, solvents and polymer used in this work. The molar volume of the different compounds was calculated using the molecular weight and the density of each compound at ambient temperature.

Table 3. Calculated group contribution Hansen Solubility parameters for all flavorants, solvents and polymer in the system

Functionality	Molecule	Solubility Parameter (δ_s) ($MPa^{0.5}$)	Vol Molar (cm^3/mol)
alcohols	cinnamyl alcohol	22.16	129.0468
	eugenol	22.57	154.9056
	citronellol	18.89	186.2807
aldehydes	ethyl vanillin	23.48	140.1096
	cinnamaldehyde	17.91	125.9026
	vanillin	21.52	144.0814
monoterpenes	menthol	19.5	175.5842
	geraniol	17.38	177.9123
	eucalyptol	16.09	167.1505
esters	menthyl acetate	11.24	212.7682
	ethyl cinnamate	18.67	168.4608
	terpinyl acetate	16.06	204.0436
polymer	HPMC	21.15	281.8153
solvents	ethyl alcohol	26.5	58.3903
	Water	47.84	18.0160

The calculated solubility parameter for the individual components were used to obtain the solubility parameters for the solvent mixtures (water:ethanol:flavorant) at the proportions used in the experiments. A linear volumetric mixing rule $\delta_s = \sum \phi_i \delta_i$ was used to obtain the value for the solvent mixture. Results are summarized on Table 4.

Table 4. Calculated Hansen solubility parameters for solvent mixtures

	Solvent mixture solubility parameter (δ_s) ($MPa^{0.5}$)					
Concentration (%) / Flavorant	**(H_2O:e-OH) (3:1)**	**0.005**	**0.01**	**0.025**	**0.05**	**0.1**
cinnamyl alcohol	41.50201	41.50113	41.50025	41.49761	41.49320	41.48439
eugenol	41.50201	41.50116	41.50032	41.49778	41.49355	41.48510
citronellol	41.50201	41.50076	41.49950	41.49575	41.48949	41.47697
menthyl acetate	41.50201	41.50129	41.50057	41.49841	41.49481	41.48762
ethyl cinnamate	41.50201	41.50094	41.49988	41.49669	41.49137	41.48073
terpinyl acetate	41.50201	41.50111	41.50022	41.49753	41.49305	41.48409
menthol	41.50201	41.50084	41.49967	41.49616	41.49031	41.47860
geraniol	41.50201	41.50069	41.49937	41.49542	41.48884	41.47567
eucalyptol	41.50201	41.50071	41.49940	41.49549	41.48898	41.47594
ethyl vanillin	41.50201	41.50047	41.49893	41.49432	41.48664	41.47127
cinnamaldehyde	41.50201	41.50098	41.49994	41.49684	41.49167	41.48134
vanillin	41.50201	41.50076	41.49950	41.49575	41.48949	41.47697

Table 4 summarizes the changes in the solubility parameters for the solvent mixtures when increasing the concentration of the different flavorants. These changes are very low in magnitude (bellow 0.04%) and are not constant; the magnitude change for the solubility parameters in the last two evaluated concentrations is in some cases up to 5 times larger. However, the effect of the addition of these small flavorant quantities have demonstrated in some cases to have a significant effect on the gelation process of HPMC as seen on previous sections in this chapter.

A good solvent for HPMC, such as water, have a high solubility parameter (47.84 $MPa^{0.5}$), as the solubility parameter decreases, the capability of solvation of the polymer decreases as well, limiting the gelation process. A synergistic effect triggered by the addition of

ethanol and flavorants as part of the solvent mixture is the main source of variation in T_{gel}, since the presence of these molecules in the solvent affect the ability of the polymer to stretch, expose the hydrophobic sites, and disturb the structures (water-polymer) that allow achieving the gelation state.

For the calculation of Flory interaction parameters (χ), the molar volume of the solvent had to be calculated. By the equation: $V_{m_s} = \sum \phi_i V_{m_i}$, where ϕ_i is the volume fraction of the different compounds in the solvent, and V_{mi}, which represents the molar volume for the *i* compounds in the solvent mixture. The molar volume for the solvents, were calculated in the range of concentration studied for all the flavorants. Results are summarized on Table 5. Changes in the molar volume when increasing the flavorant concentration are almost unnoticeable; due to solubility limitations the maximum concentration of flavorant that could be used was 0.1%,

Table 5. Calculated molar volume for all solvent mixtures

	Solvent molar volume (V_m) (cm^3/mol)					
Concentration (%) / Flavorant	**0% (H_2O:e-OH) (3:1)**	**0.005%**	**0.01%**	**0.025%**	**0.05%**	**0.1%**
cinnamyl alcohol	30.00719	30.01170	30.01621	30.02974	30.05229	30.09739
eugenol	30.00719	30.01277	30.01835	30.03509	30.06298	30.11877
citronellol	30.00719	30.01585	30.02450	30.05046	30.09372	30.18023
menthyl acetate	30.00719	30.01159	30.01598	30.02917	30.05115	30.09511
ethyl cinnamate	30.00719	30.01152	30.01584	30.02882	30.05044	30.09370
terpinyl acetate	30.00719	30.01231	30.01742	30.03276	30.05834	30.10948
menthol	30.00719	30.01494	30.02268	30.04591	30.08463	30.16205
geraniol	30.00719	30.01527	30.02335	30.04757	30.08795	30.16870
eucalyptol	30.00719	30.01423	30.02126	30.04236	30.07753	30.14787
ethyl vanillin	30.00719	30.01648	30.02576	30.05361	30.10003	30.19286
cinnamaldehyde	30.00719	30.01346	30.01973	30.03853	30.06986	30.13253
vanillin	30.00719	30.01576	30.02432	30.05002	30.09284	30.17849

Using the classical solution theory, the Flory Huggins' interaction parameters were derived as a measurement of the enthalpic and entropic contribution of the all compounds in the mixtures. Specifically using the Hansen solubility parameters for the polymer and solvent as follows

$$\chi_{i-j} = \frac{V_{m_S}}{RT}(\delta_i - \delta_j)^2 + 0.34 \quad \text{(Eq. 5)}$$

Where V_{ms} is the molar volume of the solvent mixture, R is the gas constant, T is the temperature in Kelvins, δ_i and δ_j are the Hansen solubility parameters for the polymer and solvent, respectively, and 0.34 represents a correction factor for entropic contributions.[31] As the interaction parameter, T_{gel} is also dependent on the temperature, so Flory interaction parameters for the HPMC-solvent system at 25, 50 and 75 °C were

calculated, to evaluate how it evolves during the gelation process. Results are presented in detail on the appendix. In the Figure 22, it can be observed the dependence of the interaction parameters with concentration of flavorant.

Table 6. Flory Huggins interaction parameters for the system HPMC:solvent (water:ethanol:flavorant) @25°C

Flavorant Concentration (%)	0% (H_2O:e-OH) (3:1)	0.005%	0.01%	0.025%	0.05%	0.1%
HPMC/cinnamyl alcohol	5.35665	5.35697	5.35729	5.35824	5.35984	5.36302
HPMC/eugenol	5.35665	5.35716	5.35768	5.35922	5.36180	5.36693
HPMC/citronellol	5.35665	5.35748	5.35830	5.36079	5.36492	5.37317
HPMC/menthyl acetate	5.35665	5.35703	5.35741	5.35855	5.36044	5.36423
HPMC/ethyl cinnamate	5.35665	5.35685	5.35704	5.35764	5.35863	5.36059
HPMC/terpinyl acetate	5.35665	5.35706	5.35747	5.35871	5.36077	5.36489
HPMC/menthol	5.35665	5.35736	5.35808	5.36023	5.36381	5.37095
HPMC/geraniol	5.35665	5.35735	5.35805	5.36015	5.36364	5.37060
HPMC/eucalyptol	5.35665	5.35718	5.35771	5.35931	5.36197	5.36726
HPMC/ethyl vanillin	5.35665	5.35744	5.35823	5.36061	5.36457	5.37245
HPMC/cinnamaldehyde	5.35665	5.35718	5.35772	5.35934	5.36202	5.36737
HPMC/vanillin	5.35665	5.35746	5.35828	5.36072	5.36478	5.37288

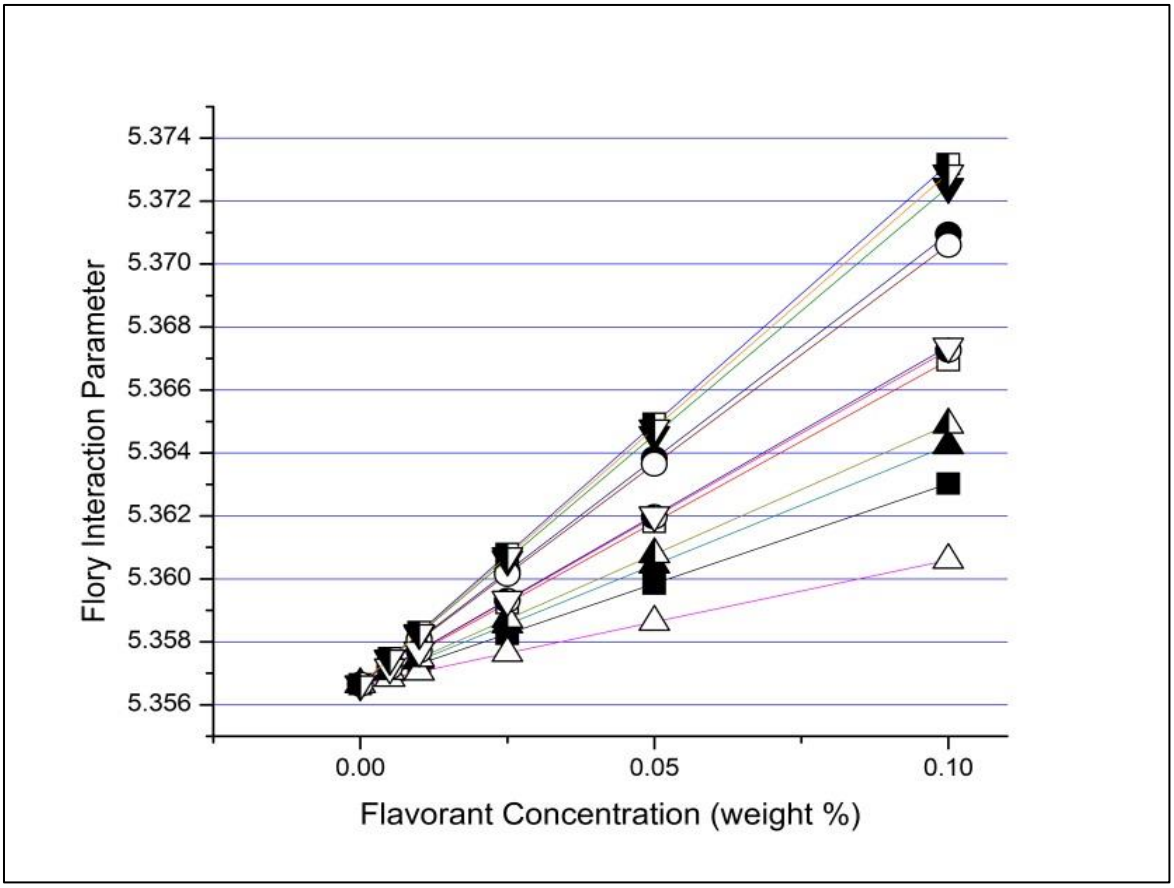


Figure 22. Flory Huggins interaction parameter calculated to all solvents (water:ethanol:flavorant) in the concentration range studied for 25°C Alcohols (squares), Esters (Triangles), Aldehydes (circles), Monoterpenes (Inverted Triangles)

The Flory's interaction parameters are proportional to the temperature of the solution and concentration of flavorant. When increasing the temperature the ability of the solvent to solvate the polymer increases, however, when increasing the quantity of flavorant in the mixture this ability decreases. As a general rule, complete miscibility is achieved when the interaction parameter is lower than 0.5 ($\chi < 0.5$), in the systems evaluated the interaction parameters are far from the ideality and the solubility of the polymer is limited, which

causes an inhibition in the structure formation stages previous to the gelation. HPMC in solution form semi-organized bundles that are highly influenced by the quality of the solvent medium. Ionic forces, hydrogen bonging and other molecular interactions contribute to the structure formation process that promotes the gel network formation. Finally, the destabilization of the water cages around the methoxyl groups in the HPMC chains is also highly dependent of the interaction between the components in the solvent mixture, and the energy barrier to break them may vary, so is T_{gel}.

2.4 Conclusions

From the measurements and results shown in this section, it can be concluded that the gelation process of HPMC solutions in presence of the flavorants evaluated is highly dependent of the type of functional group and the concentration of the flavorant molecule in the solvent mixture. Confident intervals can be set using a tolerance of $\pm 2\sigma$ for the T_{gel}, in this range of temperature the use of these flavorants as part of the formulation process can be used with little effect on the gelation process.

Esters flavorants exhibited no effect on the gelation temperature for HPMC in the studied concentration range. For a film formulation development in similar systems, these flavorants are good candidates.

The addition of flavorants in this study was proposed as a model for the evaluation of the effect of the incorporation of small soluble or partially soluble species on the gelation mechanism of HPMC. Results proved the relation between the quality of the solvent and changes in the gelation process for all samples.

2.5 References.

1. Jyoti A., Singh G., Saini S., Rana A.C. Fast Dissolving Films: A Novel Approach To Oral Drug Delivery. International Research Journal Of Pharmacy 2(12) (2011); 69 – 74.
2. Yan W., Hsiao V., Zheng Y., Shariff Y., Gao T., Huang T. Towards Nanoporous Polymer Thin Film-Based Drug Delivery Systems. Thin Solid Films 517 (2009); 1794 – 1798.
3. Ham A., Rohan L., Boczar A., Yang L., Buckheit K., Buckheit R. Vaginal Film Drug Delivery Of The Pyrimidinedione IQP-0528 For The Prevention Of HIV Infection. Pharmaceutical Research 29 (2012); 1897 – 1907.
4. Sievens-Figueroa L., Bhakay A., Jerez-Rozo J., Pandya N., Romañach R., Michniak-Kohn B., Iqbal Z., Bilgili E., Davé. Preparation And Characterization Of Hydroxypropyl Methyl Cellulose Films Containing Stable BCS Class II Drug Nanoparticles For Pharmaceutical Applications. International Journal Of Pharmaceutics 423 (2012); 496– 508.
5. Fajardo P., Martins J., Fuciños C., Pastrana L., Teixeira J., Vicente A. Evaluation Of A Chitosan-Based Edible Film As Carrier Of Natamycin To Improve The Storability Of Saloio Cheese. Journal Of Food Engineering 101 (2010); 349–356.
6. Zyck, D., Dzija M., Chapdelaine A. Edible Film Formulations Containing Maltodextrin. U.S. Patent US6740332 B2. (2004)
7. Chillo S., Flores S., Mastromatteo M., Conte A., Gerschenson L., Del Nobile M. Influence Of Glycerol And Chitosan On Tapioca Starch-Based Edible Film Properties. Journal Of Food Engineering 88 (2008); 159–168.
8. Nur Hanani Z., Beatty E., Ross Y., Morris M., Kerry J. Manufacture And Characterization Of Gelatin Films Derived From Beef, Pork And Fish Sources Using Twin Screw Extrusion. Journal Of Food Engineering 113 (2012); 606–614.
9. Jindal M., Kumar V., Rana V., Tiwary A. An Insight Into The Properties Of Aegle Marmelos Pectin–Chitosan Cross-Linked Films. International Journal Of Biological Macromolecules 52 (2013); 77– 84.
10. Lecomte F., Siepmann J., Walther M., Macrae R., Bodmeier R. B Lends Of Enteric And GIT-Insoluble Polymers Used For Film Coating: Physicochemical Characterization And Drug Release Patterns. Journal Of Controlled Release 89 (2003); 457–471.
11. Pérez P., Du W., Avena-Bustillos R., Ferreira N., Mchugh T. Edible Films From Pectin: Physical-Mechanical And Antimicrobial Properties - A Review. Food Hydrocolloids 35 (2014); 287 – 296.
12. Alsoy S., Yenal H., Ozbas B. Membrane Formation By Dry-Cast Process Model Validation Through Morphological Studies. Journal Of Membrane Science 249 (2005); 163 – 172.
13. Bartlett M. Properties Of Sufficiency And Statistical Test. Proceedings Of The Royal Society Of London. Series A, Mathematical And Physical Sciences 160 (1937); 268 – 282.

14. H. Levene. Robust Testes For Equality Of Variances. Contributions To Probability And Statistics (1960); 278–292.
15. Desbrières J., Hirrien M., Ross-Murphy S. Thermogelation Of Methylcellulose: Rheological Considerations. Polymer 41 (2000); 2451 – 2461.
16. Suzuki K., Taniguchi Y., Enomoto T. The Effect Of Pressure On Sol-Gel Transformation Of Macromolecules. Bulletin Of The Chemical Society Of Japan 45 (1972); 336 – 338.
17. Haque A., Richardson R., Morris E. Thermogelation Of Methylcellulose. Part II: Effect Of The Hydroxypropyl Substituent. Carbohydrate Polymers 22 (1993); 174 – 186.
18. Stephenson R., Stuart J. Mutual Binary Solubilities: Water-Alcohols And Water-Esters. Journal Of Chemical Engineering Data 31 (1986); 56 – 70.
19. Myagkova N., Rakhmonberdiev G., Sagdieva Z., Sidikov A. Thermodynamic Properties Of Solutions Of Water-Soluble Mixed Cellulose Esters. Chemistry Of Natural Compounds 33(1) (1997); 76 – 79.
20. Thomson R. The Chemistry Of Natural Products, Second Edition. Springer-Science + Business Media. Pag 107. (1993).
21. Trombetta D., Castelli F., Sarpietro M., Venuti V., Cristani M., Daniele C., Saija A., Mazzanti G., Bisignano G. Mechanisms Of Antibacterial Action Of Three Monoterpenes. Antimicrobial Agents And Chemotherapy 49 (6) (2005); 2474 – 2478.
22. Monoterpene Properties. Merck Index Online. Https://Www.Rsc.Org/Merck-Index/
23. Lide D. Ed. CRC Handbook Of Chemistry And Physics (2005). CRC Press, Boca Raton, Fl. Monoterpene Solubility.
24. Weidenhamer J., Macias F., Fischer N., Williamson B. Just How Insoluble Are Monoterpenes?. Journal Of Chemical Ecology 19(8) (1993); 1799 – 1807.
25. Hansen C. The Three-Dimensional Solubility Parameter And Solvent Diffusion Coefficient. Danish Technical Press. Copenhagen (1967).
26. Hansen C. Hansen Solubility Parameters – A User's Handbook. CRC Press, Boca Raton, Fl. (2000).
27. Haque A., Morris E. Thermogelation Of Methylcellulose. Part I: Molecular Structures And Processes. Carbohydrate Polymers 22 (1993); 161 – 173.
28. Hirrien M., Chevillard C., Desbrières J., Axelos M., Rinaudo M. Thermogelation Of Methylcelluloses: New Evidence For Understanding The Gelation Mechanism. Polymer 39(25) (1998); 6251 – 6259.
29. Van Krevelen D., Te Nijenhuis K. Properties Of Polymers, Fourth Edition. Chapter 7 - Cohesive Properties And Solubility. Elseiver, (2009).
30. Brandrup J., Immergut E., Grulke E. Polymer Handbool, Fourth Edition. Volume 2. Table 4, Page 686. Wiley-Interscience (1999).
31. Emerson J., Toolan D., Howse J., Furst E., Epps T. Determination Of Solvent−Polymer And Polymer−Polymer Flory−Huggins Interaction Parameters

For Poly(3-Hexylthiophene) Via Solvent Vapor Swelling. Macromolecules 46 (2013); 6533 – 6540.

3. EFFECT OF THE POLYMER MOLECULAR WEIGHT ON TGEL (ADDITION OF BCS-CLASS II DRUGS)

3.1 Introduction

In the development and design of a drug delivery product, the solubility and the interactions of the active pharmaceutical ingredient (API) particles, with the rest of the formulation components is a key factor to take into account. A special case occurs when the pharmaceutical product has low water solubility, such as Biopharmaceutical Classification System (BCS) class II drugs, characterized by their poor water solubility and high permeability in the human body.[1] Permeability across biological membranes is a main factor in the absorption and distribution of drugs. Poor permeability can arise due to a number of structural features and membrane-based efflux mechanisms.[2] It can lead to poor absorption across the gastrointestinal mucosa or poor distribution throughout the body. Moreover, for BSC II drugs, the low solubility in aqueous and biological mediums reduces significantly the efficiency in the drug transport and bioavailability.[3, 4]

Particle size reduction is usually an approach used to increase the solubility of these APIs in aqueous or biological mediums.[5-7] Nevertheless, in order to enhance the bioavailability it is essential to preserve the particle size after the drug administration. Since it is extremely common to observe agglomeration of particles due to their large surface area, the colloidal stability of these particles has to be controlled during

processing. [8-10] Agglomeration does not only affect the bioavailability of the drug, but also decreases its permeability, reducing the drug's effectivity.[11]

In order to overcome these limitations, encapsulation and stabilization systems are proposed as methods for improving the bioavailability of poor soluble drugs by exploiting the ability of a biopolymer matrix to trap drug particles within a gel network without incurring in higher cost due to pretreatments. While forming a gel, in the case of the thermotropic hydroxypropyl methylcellulose matrix, an increase in the temperature of the polymer solution is necessary to promote interactions between the polymer chains and solvent which affects the stability of the water cages around the hydrophobic sites of the polymer chains. These interactions promote the gelation of the polymer.

In this study griseogulvin (GF) and fenofibrate (FNB) were selected as API of interest, since these drugs have very low solubility in aqueous media. The particle size was not varied or controlled, since the APIs are proposed to be used without further treatment eliminating the cost and time spent on pretreatments. The focus is to evaluate the effect of the concentration of these hydrophobic drugs on the gelation process of the polymer matrix due to interactions between the polymer and the drug particles, the solubility of the drug (FNB is almost seventeen times less soluble than GF), and how these interactions are associated to the molecular weight (chain length) of the chosen polymer. Although it is known that there is an effect by the particle size, in this study is not considered since it

have been reported that the difference in average particle size for GF and FNB is less than 2μm.[12]

3.2 Experimental section

3.2.1 Materials

Hydroxypropyl methylcellulose (HPMC) (CAS-No: 9004-65-3) grade E4M and grade E15LV (CAS-No: 9004-65-3) were purchased from Sigma-Aldrich. Griseofulvin (GF) (CAS-No: 126-07-8) from Alfa Aesar and fenofibrate (FNB) (CAS-No: 49562-28-9) from Sigma-Aldrich were used without any modification. Deionized water was used as the solvent. The physicochemical properties of the evaluated drugs are summarized on Table 7.

Table 7. Physicochemical Properties of the drugs

Drug	Solubility (mg/L)	Molecular weight	Melting Point (°C)	Log P
Griseofulvin	8.99	352.8	220	3.5
Fenofibrate	0.50	360.8	80.5	4.4

LogP is the logarithm of the partition coefficient. The partition coefficient is a ratio of the concentrations of an un-ionized compound between two liquid phases. For GF and FNB the two phases is water and the other is a non-polar solvent, in this case LogP is also

known as a measure of lipophilicity (affinity to dissolve in fats, oils, lipids and non-polar solvents).

HPMC E4M and HPMC E15LV have similar substitution degree for methoxy and hydroxypropyl substituents (Table 8). However, the main difference lies in the chain length or number of repetitive units (degree of polymerization). From the nomenclature for HPMC products (or methoxel), E represents a hydroxypropyl methylcellulose product, 4 represents the viscosity in mPa s and the letter M denotes 1000. Which identify the product as a Hydroxypropyl methylcellulose viscosity grade 4000 mPa s. "LV" denotes "*Low viscosity*" for the E15LV product.

Table 8. Physicochemical properties of HPMC polymers (data provided by the manufacturer)

Viscosity grade	Mn	Viscosity (mPa.s) (2% in H2O @25°C)	% methoxy substituents	%hydroxypropyl substituents
E4M	86kDa	3500 – 5600	28 – 30%	7 – 12%
E15LV	~6kDa	~15	28 – 30%	~9%

3.2.2 Sample Preparation & Characterization

HPMC grade E4M 2% (weight %) and HPMC grade E15LV 4% (weight %) stock solutions were prepared. Deionized water was heated to 80 °C while stirred. When the water reached the desired temperature, the HPMC was incorporated in small quantities allowing complete dissolution. When the polymer was incorporated into solution, the

temperature was reduced to 35 °C and the agitation was maintained from 24 to 36 hours until the solution turned completely clear. The desired amount of GF and FNB was weighed and added to a dilution of the stock HPMC solution to achieve the desired concentration (1% and 2% for E4M and E15LV, respectively). The resulting polymer/drug solutions were magnetically stirred for at least 12 hours guarantee homogenous dispersion of the drug particles in the polymer solution. Concentrations from 0 to 5% (weight) of E4M solutions and 0 to 10% for E15LV were prepared to maintain same drug/polymer ratio.

In this chapter the effect of the addition of GF and FNB to the gelation temperature of HPMC E4M and E15LV solutions in concentrations corresponding from 0 to 5% *drug/polymer* ratio was studied. Since solutions with HPMC E15LV at 1% shown very low viscosity (<2cP), and due to equipment sensitivity, the E15LV solutions were prepared at twice the concentration of the E4M solutions to compensate as stated before.

Rheological measurements with the same parameters as those described on Chapter 2 were performed to determine the gelation temperature of the samples. An ATS Rheologica Stresstech HR (stress-controlled) rheometer equipped with an ETS temperature controller and a double-gap Couette fixture was used for these measurements. All measurements were performed in triplicate and results correspond to the average ± the standard deviation.

3.3 Results and discussion

3.3.1 Effect of the drug concentration

In this section, the effect of the addition of the drug and its concentration in the gelation process and gelation temperature of HPMC solutions (E4M) was studied. Two different drugs were evaluated, in the concentration range from 0 to 5 drug/polymer ratio.

BCS class II drugs used in this section has solubilities in a range from 0.5 – 9 mg/L, hereby at high concentrations it can be some agglomeration and precipitation. This behavior was observed in GF solutions above 5% and will be discussed later.

In Figure 23 it can be observed that when increasing the drug concentration there is a reduction on the gelation temperature of the solution. This phenomenon was explained in a previous publication from our group [13], and was attributed to the adsorption of polymer on the drug particle's surface by hydrophobic interaction, which works as a bridge to enhance the gelation process while it decreases bulk viscosity of the sample due to depletion. Polymer-particle hydrophobic interactions reduce the necessary energy to form a gel matrix. However, the morphological (and mechanical) properties of these gels are affected by the particle concentration.

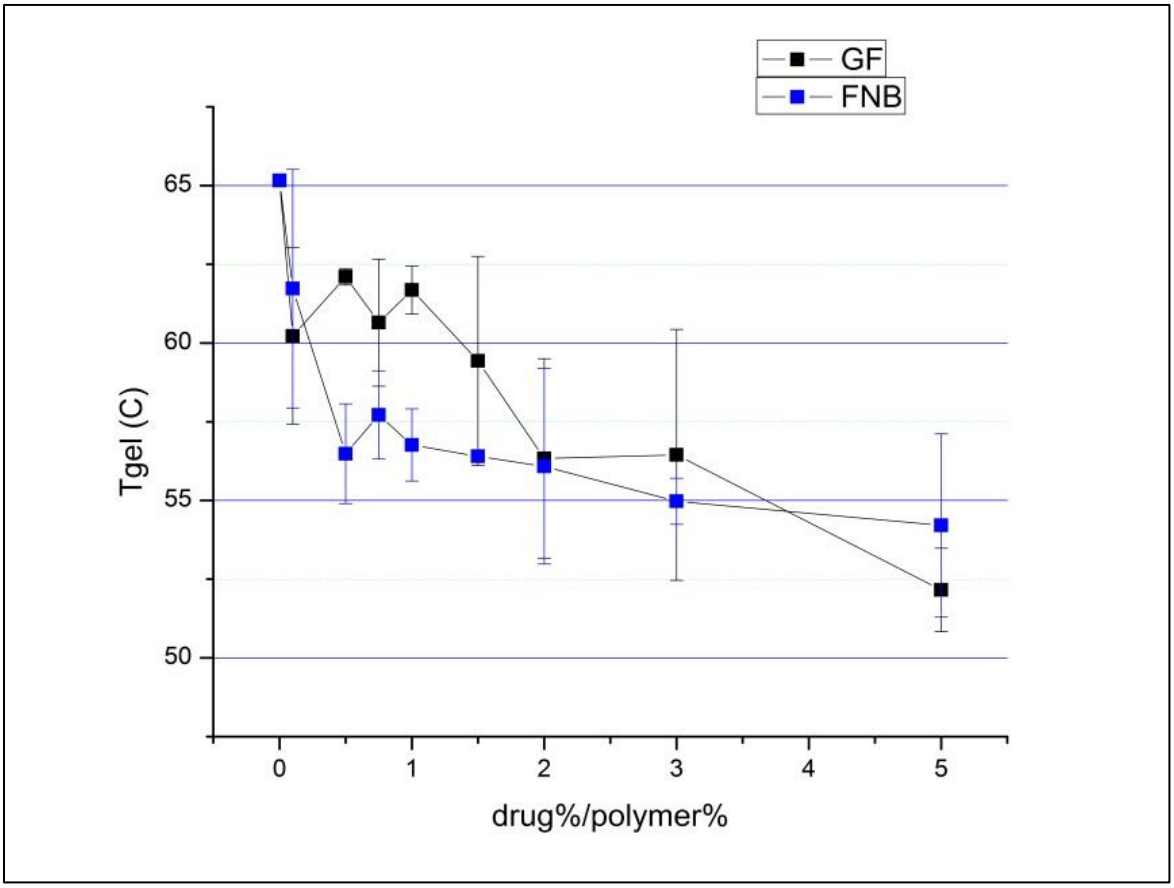


Figure 23. Effect of the concentration of BCS class II drugs on the gelation temperature (T_{gel}) of HPMC E4M solutions

It can be observed on the Figure 23 a decrease in T_{gel} in concentrations as low as 0.1 drug/polymer ratio. A linear relation between drug/polymer ratio and T_{gel} is observed for GF and FNB above 0.5% concentrations. HPMC in presence of GF exhibit a more strong dependence of the drug concentration, while in presence of FNB the main change is observed in the lowest concentration when a significant reduction of T_{gel} was observed, after that initial saturation the system changes in T_{gel} are statistically insignificant (see appendix).

For GF in HPMC E4M solutions it was observed in additional experiments, that at concentrations above 5%, the gel matrix collapses and the drug agglomerates at the bottom of the container. This occurs because the weight of the particles when aggregated is stronger than the interaction forces between the polymer chains and the particles. This behavior can be observed the Figure 24 and 25. After the gelation, an increase in the storage and loss moduli occurs (as well the viscosity), however at higher temperatures, the interactions between polymer chains fail to stabilize the particles and a drop in both moduli is observed. After the gel collapses, the particles precipitate and a interested behavior in G' and G" is observed.

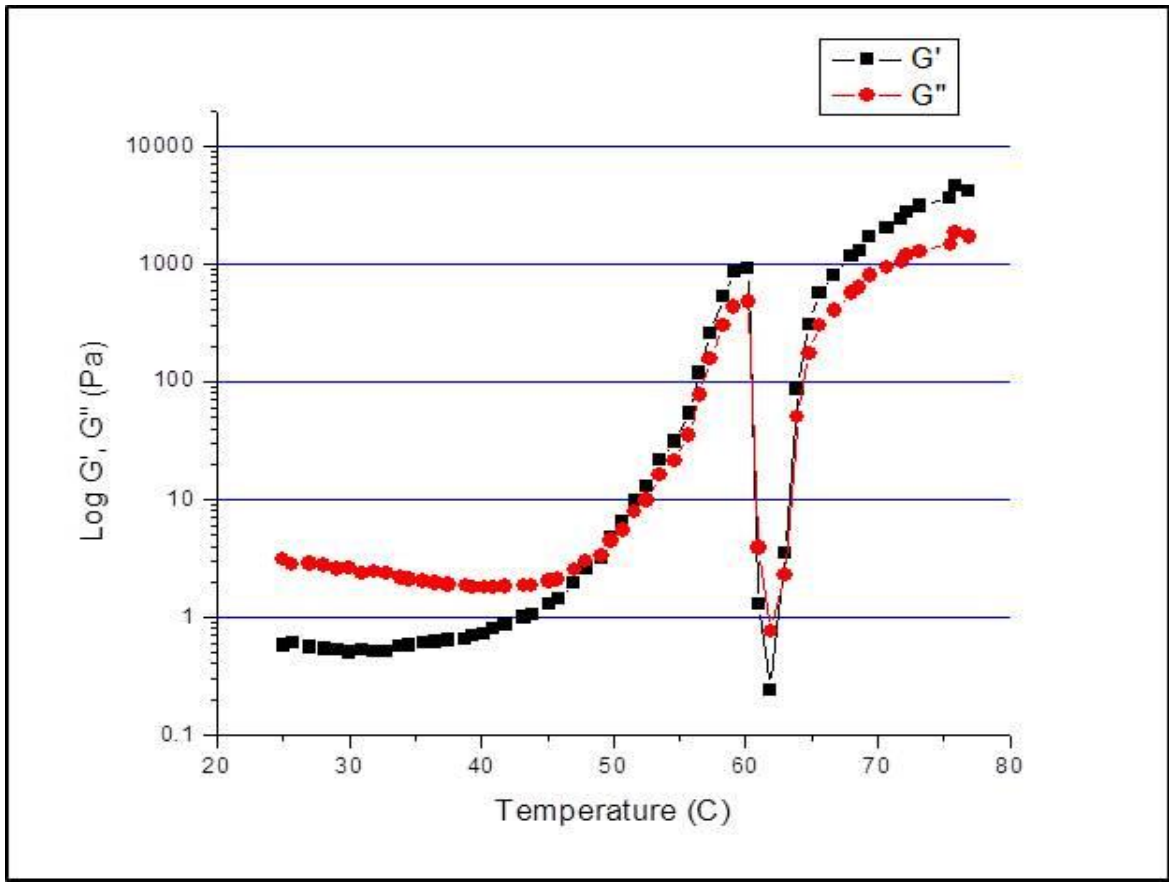


Figure 24. HPMC E4M – GF 7% gel failure measurement – oscillatory shear test

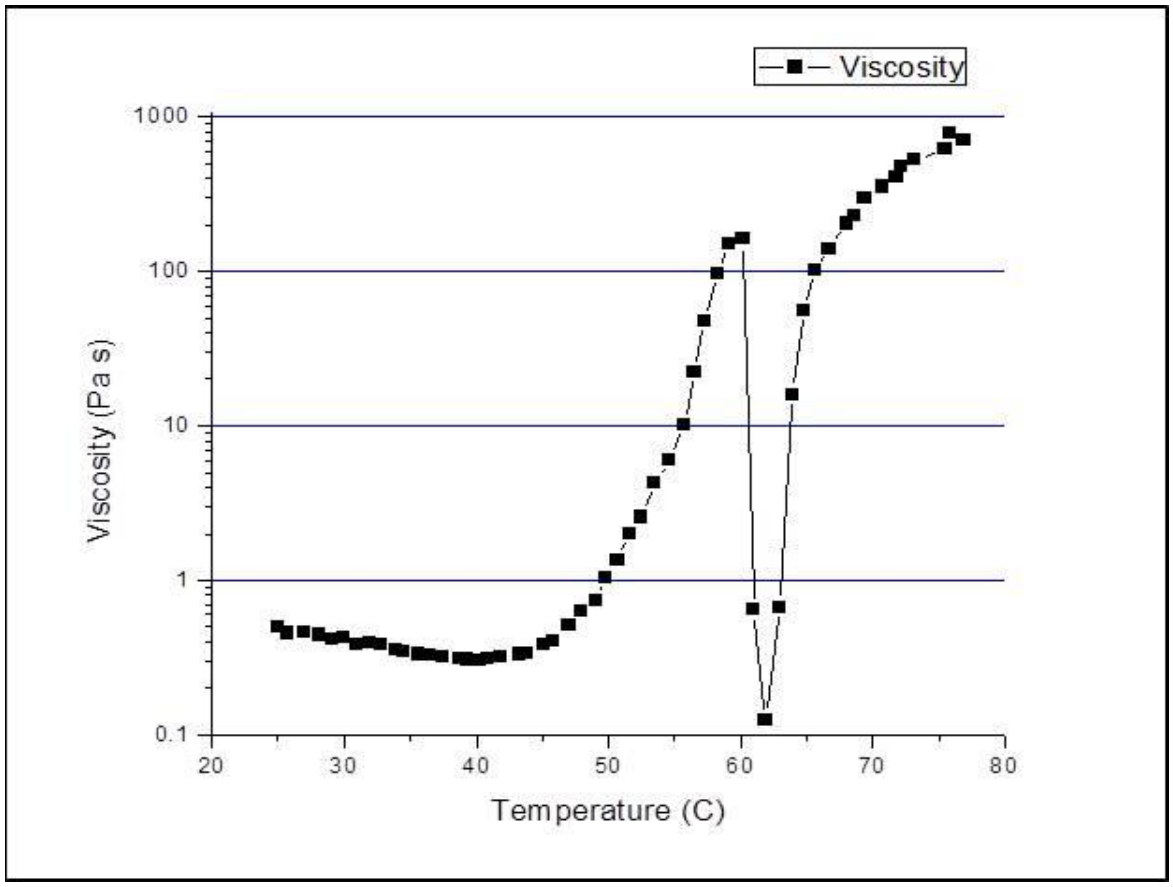


Figure 25. HPMC E4M – GF 7% gel failure measurement – oscillatory shear test

This phenomenon was corroborated by visual inspection of the suspension (Figure 26). A solution of HPMC E4M 1% - GF 7% by weight was prepared and stored in a clear container, then was heated slowly in a Themo Precision 655 convection oven from 25 to 80 °C at 5 °C steps every five minutes, followed by an additional five minutes for stabilization. Until 40 °C, a uniform dispersion was observed. Above 50 °C a phase separation is observed where the GF particles precipitate to the bottom of the container.

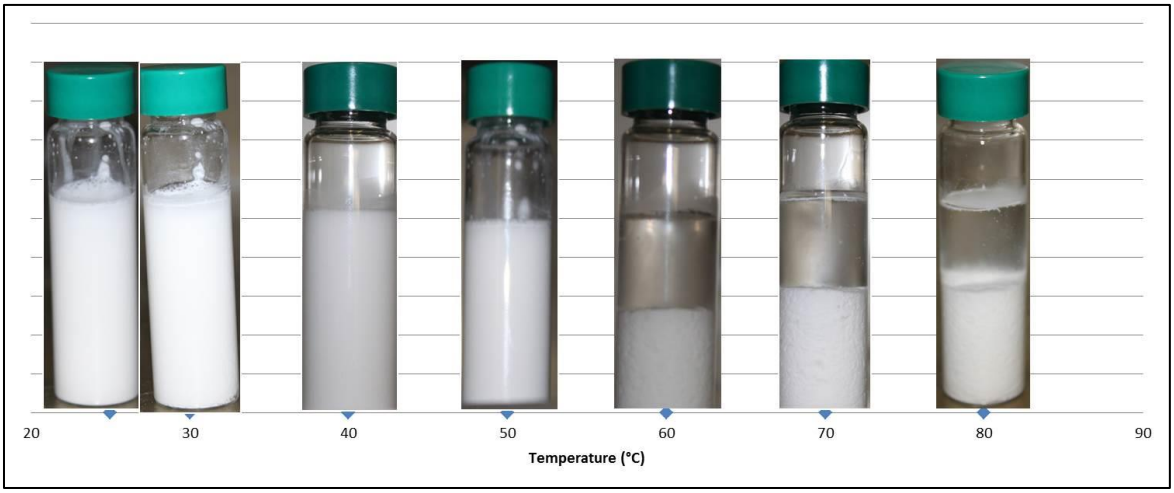


Figure 26. Gelation and failure of HPMC E4M 1% - GF 7% solution

Statistical analyses were used to support the results of the effect of the drug concentration on the gelation temperature of HPMC solutions. P-values below 0.001 for GF and FNB in the concentration range studied were obtained confirming the significant effect of the addition of these particles on the gelation temperature of HPMC solutions. Statistical analysis is discussed with more detail on the appendix for this section.

3.3.2 Effect of the polymer molecular weight

Gelation mechanism of HPMC is based on two different stages of structure formation. Initially polymer chains form bundles of molecules, semi-organized, with an increase in the medium temperature this bundles start to separate and then water-cages structures are formed around the hydrophobic (more non-polar) groups in the polymer structure. In the second stage these water cages are disrupted by an increase in the entropy of the system

(due the high temperature) and the hydrophobic groups are exposed. Finally, hydrophobic interactions between polymer chains complete the gel network formation. This process is highly related to the quality of the solvent, degree of substitution (for both hydroxypropyl and methoxil groups), concentration, temperature, the presence of other solutes, and chain length (molecular weight).[14]

In this section the effect of the polymer molecular weight in the gelation temperature of HPMC solutions in presence of two different BCS Class II drugs is evaluated. HPMC solutions for E4M and E15LV viscosity grade were prepared with concentration of GF and FNB up to 5% (drug/polymer ratio).

For GF the effect of the molecular weight is significant in the studied concentration range. Figure 27 show the behavior of the different HPMC solutions when increasing the drug concentration.

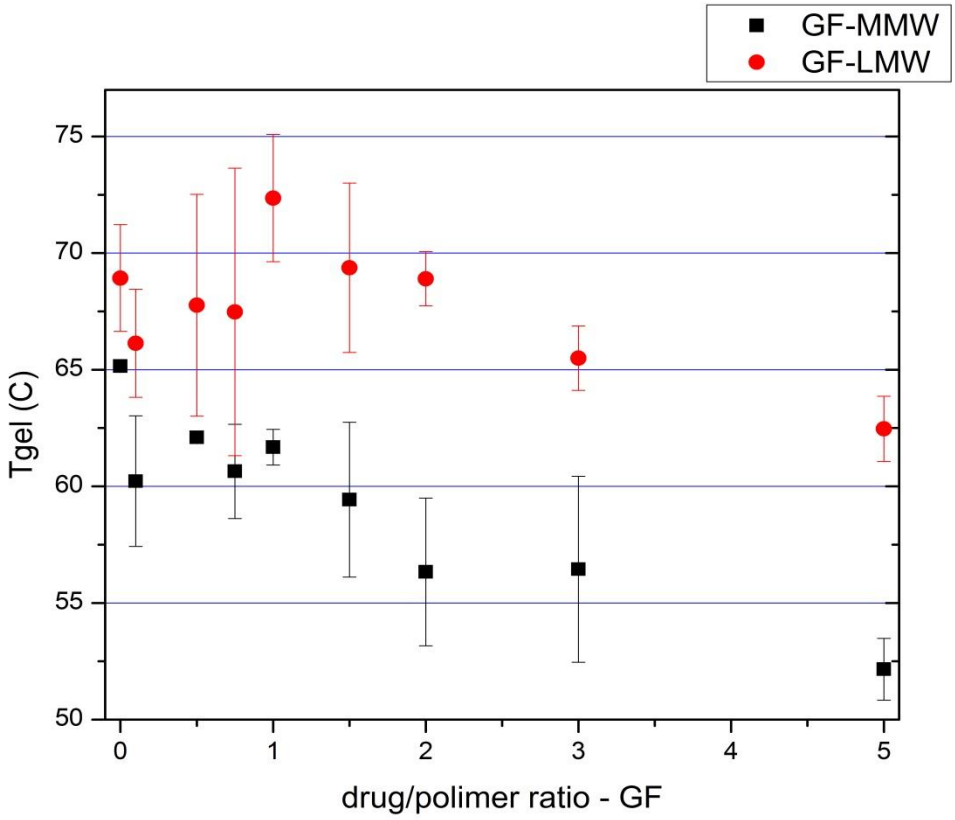


Figure 27. Effect of the molecular weight for HPMC-griseofulvin solutions

A clear difference in the gelation temperatures for al griseofulvin concentrations is observed. However, T_{gel} does not follow a common trend for both molecular weights. For fenofibrate (Figure 28) similar behavior is observed.

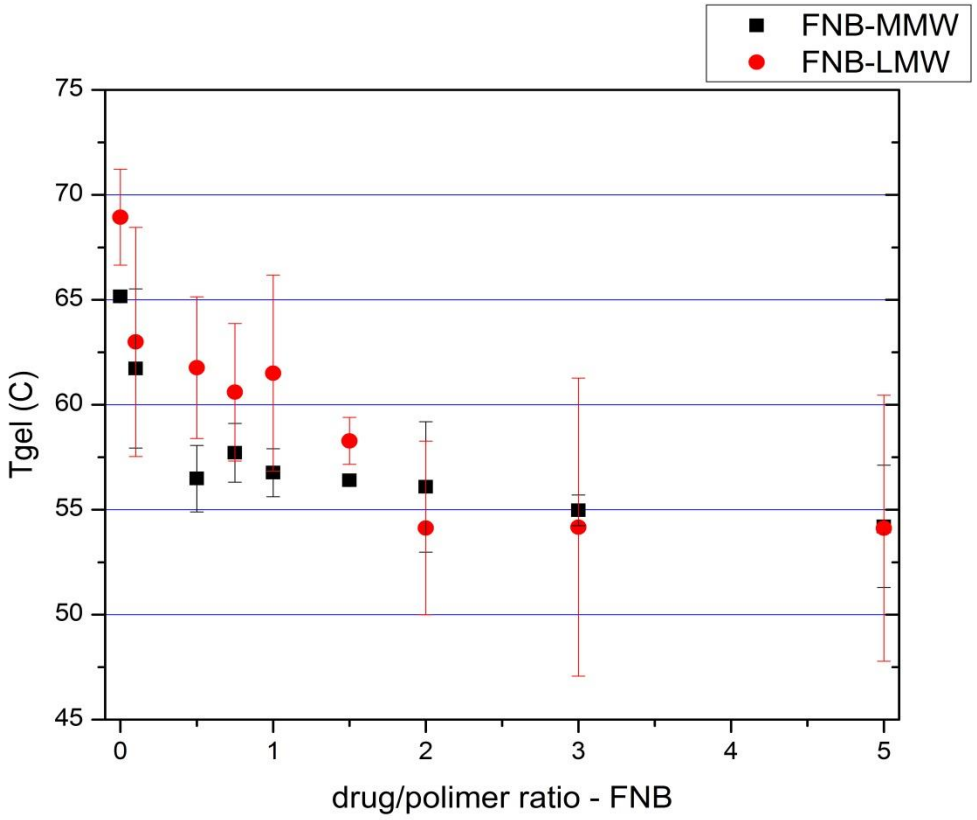


Figure 28. Effect of the molecular weight for HPMC-fenofibrate solutions

The strong interactions between the polymer and the hydrophobic sites in the drug surface were previously reported as a main factor in the network formation for HPMC solutions in the presence of a highly hydrophobic drug particle.[13] As stated before, the polymer chains serve as bridges between drug particles; these structures require that the polymer has sufficient hydrophobic substitutions to form bonds with both particles. Polymer with a higher molecular weight statistically has more hydrophobic substitution than a polymer with a smaller chain. While the polymer with a longer chain can interact with more drug

particles, a small chain polymer may be adsorbed totally on the surface of one particle, neglecting the bridge structure formation.

The storage modulus of the polymer solutions is related to the amount of crosslinks between polymer molecules and other molecules or in this case, nodes of polymer molecules attached on the drug surface. Figure 29 show the behavior of this modulus when increasing the temperature (network formation stage) for both molecular weight polymers.

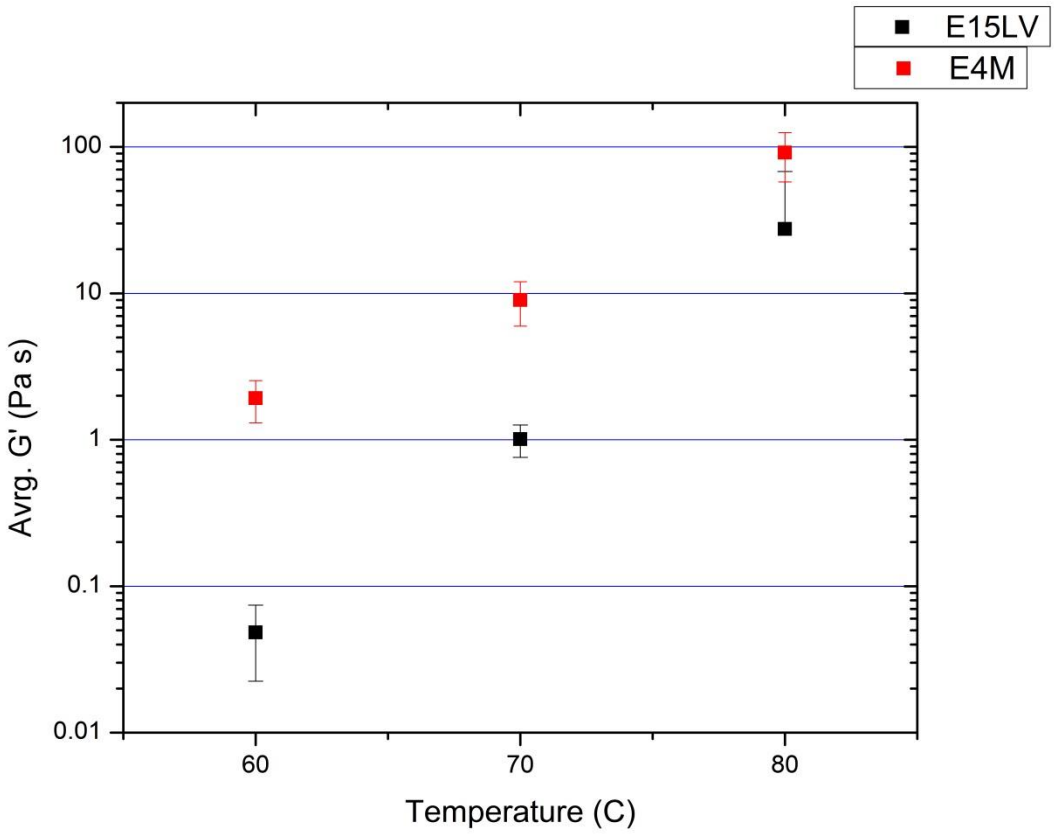


Figure 29. Storage modulus for HPMC E4M and E15LV in the last stage of gel formation

Romanski et al (2011) and Zhu et al (2011), reported by using computer simulations, the average interfacial binding energy for HPMC on the surface of GF (-180kcal/mol/nm^2) and FNB (-150kcal/mol/nm^2) crystals.[15,16] and concluded that HPMC have a strong adsorption on GF and FNB molecules due to its relatively long hydrophobic branches (~6Å), which could potentially provide significantly strong van der Wall forces with the drug hydrophobic surface. However, this adsorption phenomenon is highly influenced by the chain length; small polymer molecules can be adsorbed almost completely flat on the drug surface, while long polymer chains can be adsorbed partially leaving enough hydrophobic substitutions free to form entanglements with other polymer molecules or other particles.

A direct relation between the radius of gyration and the molecular weight of the polymer was proposed by Flory [17] and represent the dimensions of a polymer chain, or the end to end distance in the molecule. This value depends on the quality of the solvent, temperature of the medium and degree of polymerization of the polymer (molecular weight).

$$R_g \sim N^v$$

Where R_g is the radius of gyration, N represents the number of bond segments (degree of polymerization) and v is the Flory exponent for the solvent quality. A good solvent for HPMC as water has a v value of 1/3. This value (R_g) can be related to the amount of crosslinks or interactions that a polymer chain has the ability to do in a good solvent media. For HPMC E4M and E15LV a relation can be obtained as follows.

$$\frac{R_{g_{E4M}}}{R_{g_{E15LV}}} = \frac{N_{E4M}^{0.6}}{N_{E15LV}^{0.6}}$$

$$\frac{R_{g_{E4M}}}{R_{g_{E15LV}}} = \left(\frac{M_{E4M}}{M_{E15LV}}\right)^{0.6}$$

For E4M and E15LV, the values of *M* are 86kDa and 6kDa respectively. Replacing these values on the previous equation an expression for compare the Rg for both polymers can be obtained.

$$\frac{R_{g_{E4M}}}{R_{g_{E15LV}}} = \left(\frac{86}{8}\right)^{0.6} = 4.93$$

$$R_{g_{E4M}} = 4.93\, R_{g_{E15LV}}$$

E4M suspended in an aqueous solution has almost 5 times the extent of E15LV, hence is capable of form more interactions with other polymer molecules or drug particles, reducing T_{gel} in the same conditions evaluated, further calculations were made to achieve a more deep understanding of these interactions.

The effect of the polymer molecular weight on the gelation temperature of HPMC solutions with griseofulvin concentrations up to 5% (%drug/%polymer), seems to be

related to the amount of hydrophilic polymer-polymer interactions that a polymer absorbed to the surface of the hydrophobic drug has with other free or absorbed polymer molecules. The amount of hydrophobic links between polymers and drug particles are also related to the strength of the gel and its flexibility, since these interactions have a significant effect on the microstructure of the system.

The amount of interactions that a polymer molecule can make is directly proportional to the amount of hydrophobic groups on its structure. A smaller polymer chain (lower radius of gyration, R_g) has less hydrophobic substitutions, hence is capable of less interactions with other polymer molecules. Furthermore, the effect of the addition of hydrophobic particles, with high superficial area reduces these polymer-polymer interactions since the polymer is absorbed or partially absorbed on the particle surface. A competitive effect is observed between R_g and the interparticle spacing, which is a limiting factor of the polymer-polymer interactions that stimulate the gel formation.

Rheological measurements for HPMC E4M and E15LV with concentrations up to 5% (%drug/%polymer) were performed to determine the gel strength at 80 °C (temperature at which the gel is completely formed). The elastic modulus G' was monitored when increasing the angular frequency to determine the gel strength and its relation with drug concentration in solution.

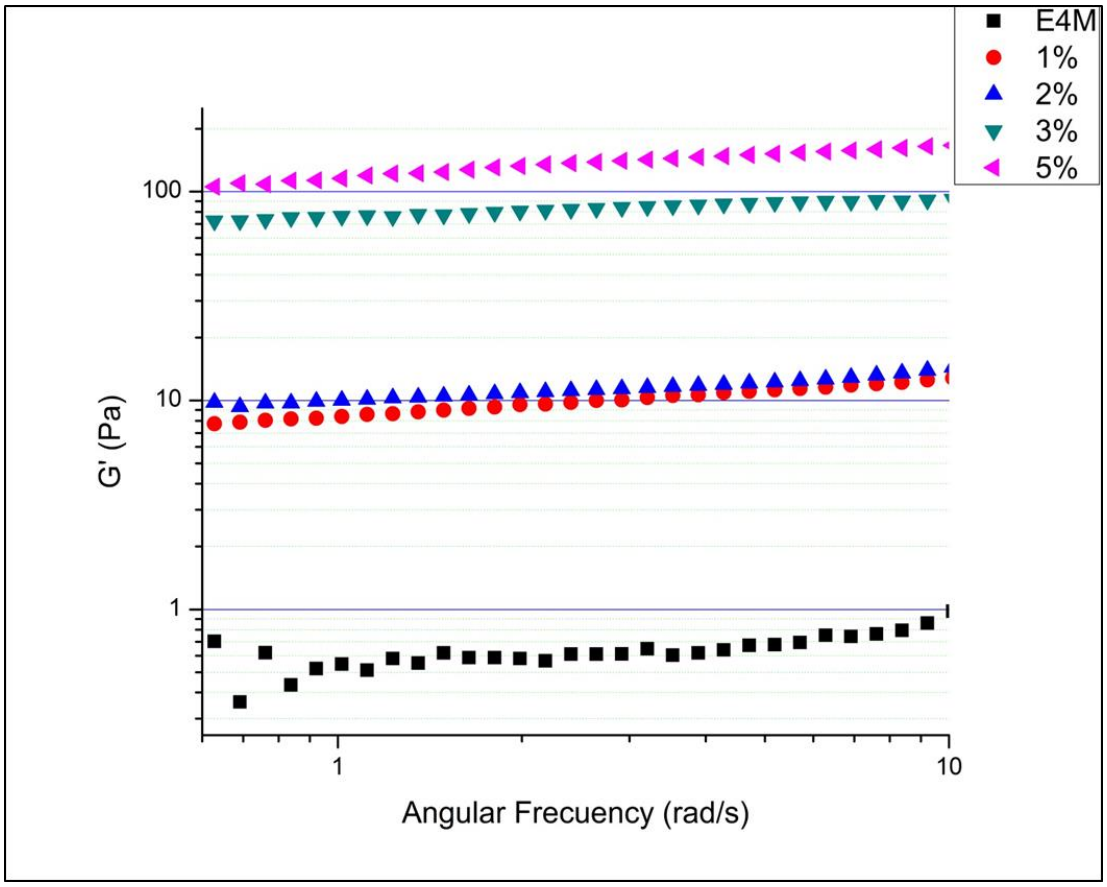


Figure 30. Elastic modulus dependence with angular frequency for HPMC E4M – Griseofulvin mixtures

In Figure 30, it can be observed that for the E4M polymer, when increasing the drug concentration in the solution, the gel strength increases. A different behavior for HPMC E15LV solutions was observed, when increasing the GF concentration the gel strength increases but at 6% by weight of particles the value for G' decreases below the value reported for 4% (Figure 31).

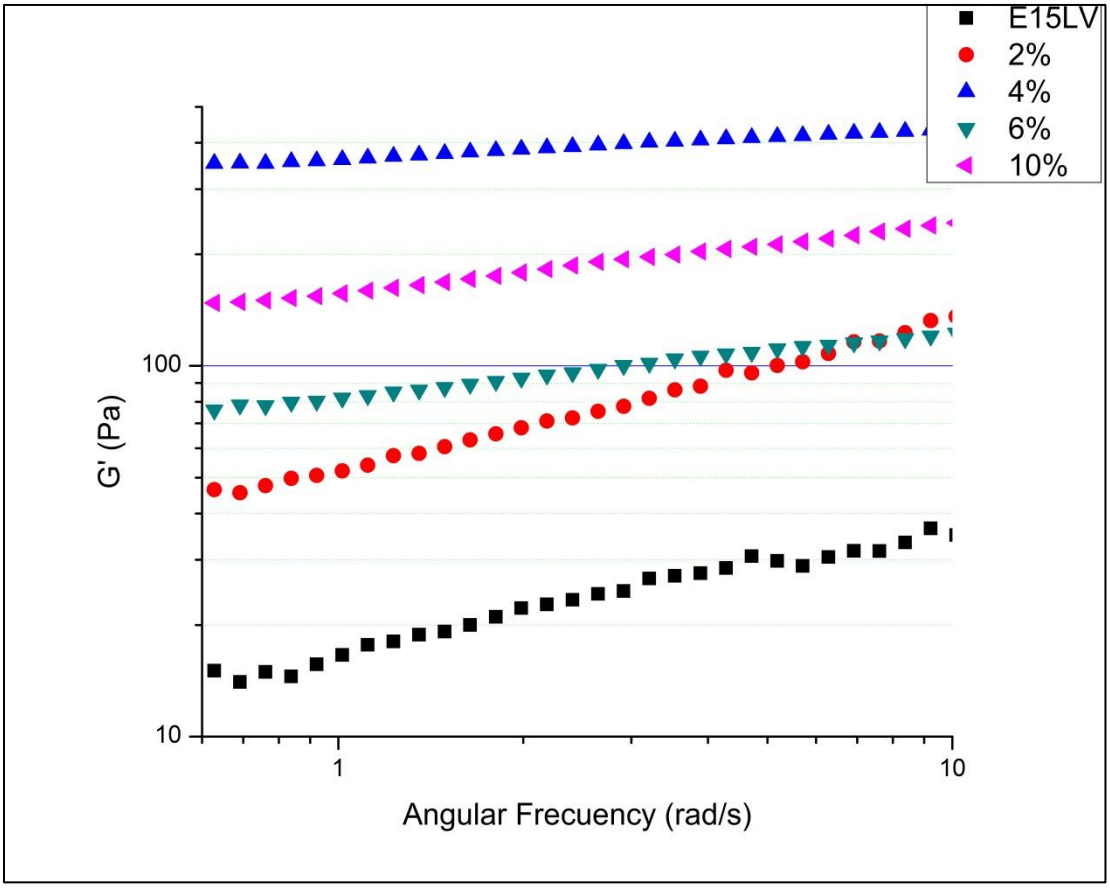


Figure 31. Elastic modulus dependence with angular frequency for HPMC E15LV – Griseofulvin mixtures

These variations can be attributed to the competing effect of the absorption of the polymer on the drug's surface and the interactions between polymer chains to form the network. Calculations of the interparticle distance were compared to the R_g of the polymer, which represents the effective length of the molecules to interact with other polymer molecules in the solution. For HPMC E4M a R_g of 35 nm have been reported [18], and since $R_{g_{E4M}} = 4.93\ R_{g_{E15LV}}$ the Rg for E15LV is 7.0 nm approximately.

The interparticle space (IPS) for GF in the polymer solution can be calculated as a function of the particle size, volume fraction, and density of the drug. For 1% GF in an HPMC E4M solution the IPS can be calculated as follows:

$$IPS = 2r\left[\left(\frac{\phi_m}{\phi}\right)^{1/3} - 1\right] for\ \phi \ll \phi_m \text{ [19]}$$

Where ϕ_m is the maximum volume fraction for hard spheres in solution (0.52), ϕ is the drug volume fraction and r is the particle radius. For 1% GF in 50 ml of solution, ϕ = 0.0071 and r = 5.9 μm, then $IPS = 36.87\mu m$.

For different concentrations of GF in both E4M and E15LV solutions, calculations for IPS were made. The results are shown on Table 9 and 10.

Table 9. Interparticle space for GF in HPMC E4M solutions

% GF	1	2	3	5
ϕ	0.007	0.014	0.022	0.037
IPS(μm)	36.87	28.84	22.00	16.76

Table 10. Interparticle space calculations for GF in HPMC E15LV solutions

% GF	2	4	6	10
ϕ	0.014	0.029	0.044	0.076
IPS(μm)	26.84	18.94	15.09	10.91

When the IPS is equal or lower than two times the particle radius (Figure 32), electrostatic and steric interactions between drug particles begin to significantly affect the system dynamics. For HPMC E4M, the IPS for all concentrations is higher than the particle diameter, but for HPMC E15LV concentrations above 6% exhibited this behavior.

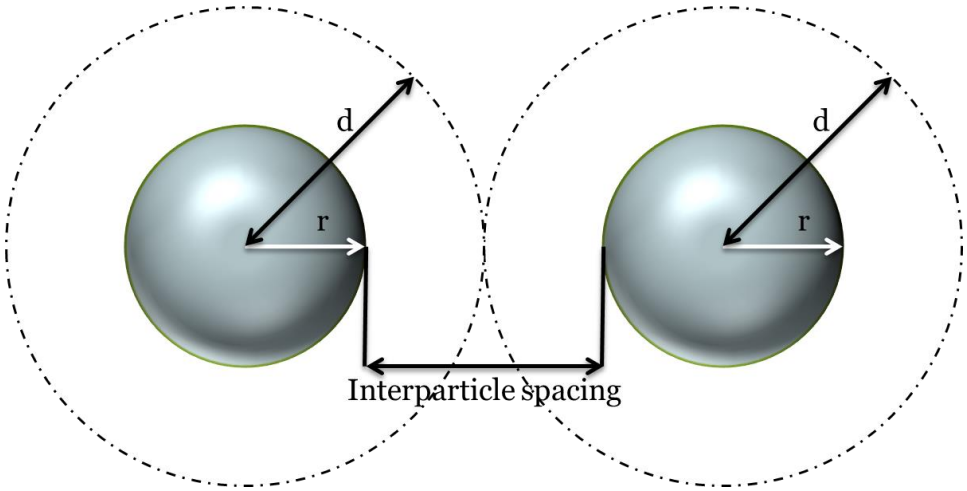


Figure 32. Graphical representation of the minimum interparticle spacing between two GF particles in solution.

HPMC E4M is capable of absorbing on the surface of the particle by hydrophobic interactions. A partially absorbed polymer molecule is able to interact with other free polymer molecules or with other drug particles, forming bridges between particles, and stimulating the complex formation that promotes the formation of the tridimensional network (Figure 33).

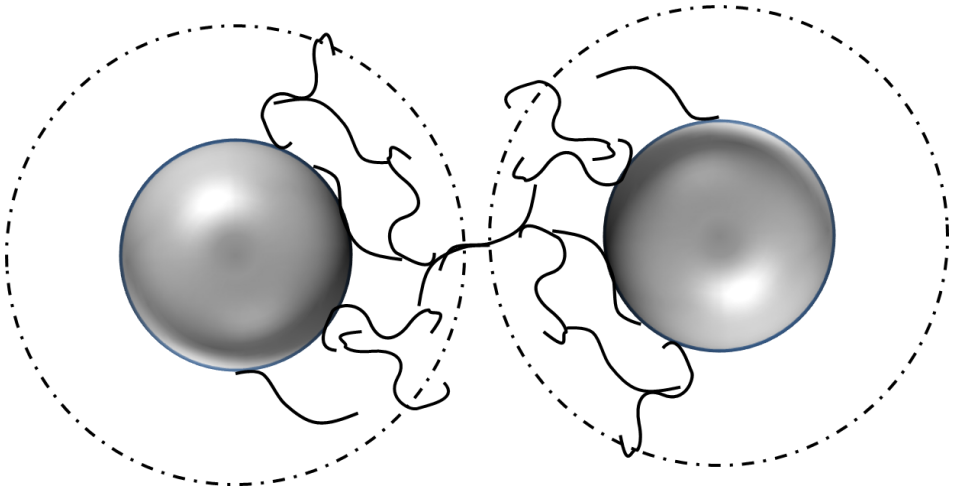


Figure 33. Bridge structure formation between two GF particles with HPMC absorbed.

For HPMC E15LV, the chain length is the limiting factor. Since the R_g of E15LV is significantly small, a complete absorption of the polymer molecules on the particle surface may occur limiting the interactions between the polymer chains due to the lack of free hydrophobic groups in the absorbed molecule. As shown on the Figure 34, the small HPMC molecules, and the high superficial area provided by the drug particles (in this case up to 10% by weight in solution), generate a limiting synergistic effect on the polymer-polymer interactions, increasing the gelation temperature and reducing the gel strength.

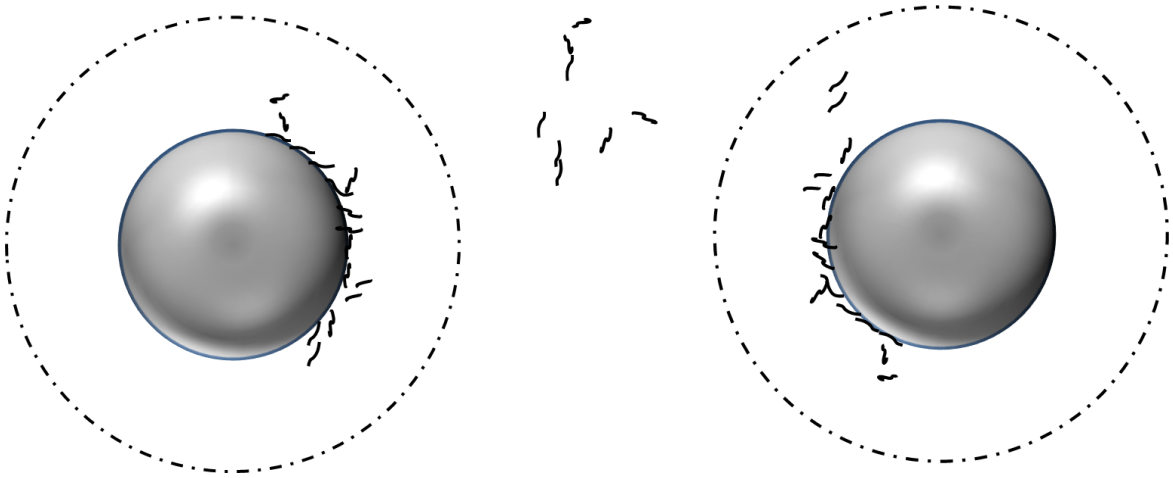


Figure 34. HPMC E15LV absorption on GF particle surface. Limiting effect on the gelation process of HPMC.

Loss tangent (tanδ) vs frequency plots were constructed for both E15LV and E4M HPMC polymer solutions, with the complete concentration range of GF. tanδ represents the ratio between the viscous modulus (G") and the elastic modulus (G'); the lower the value of tanδ a more “solid-like” behavior has the sample. For both E4M and E15LV HPMC solutions values bellow 0.4 were obtained, however as expected, for E15LV tanδ exhibited larger values, as seem on Figures 35 and 36.

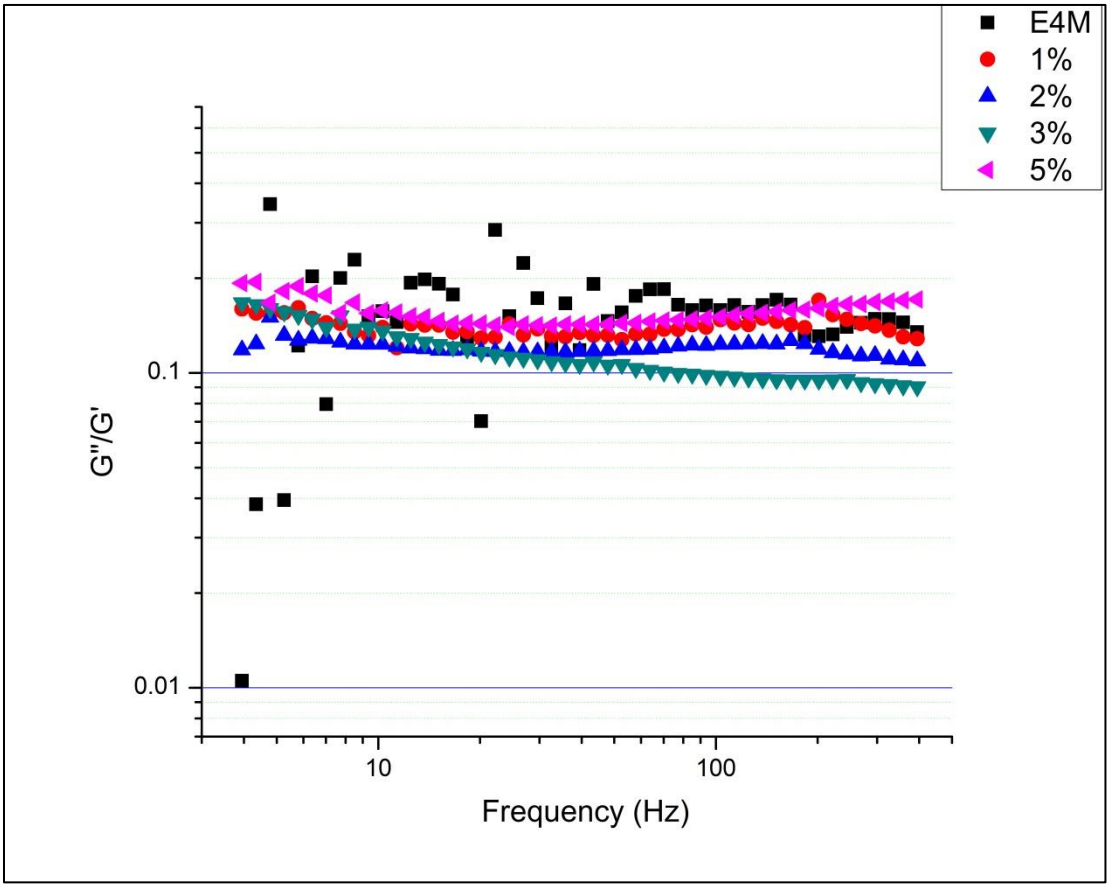


Figure 35. Viscoelastic behavior of HPMC E4M – GF solutions @80 °C

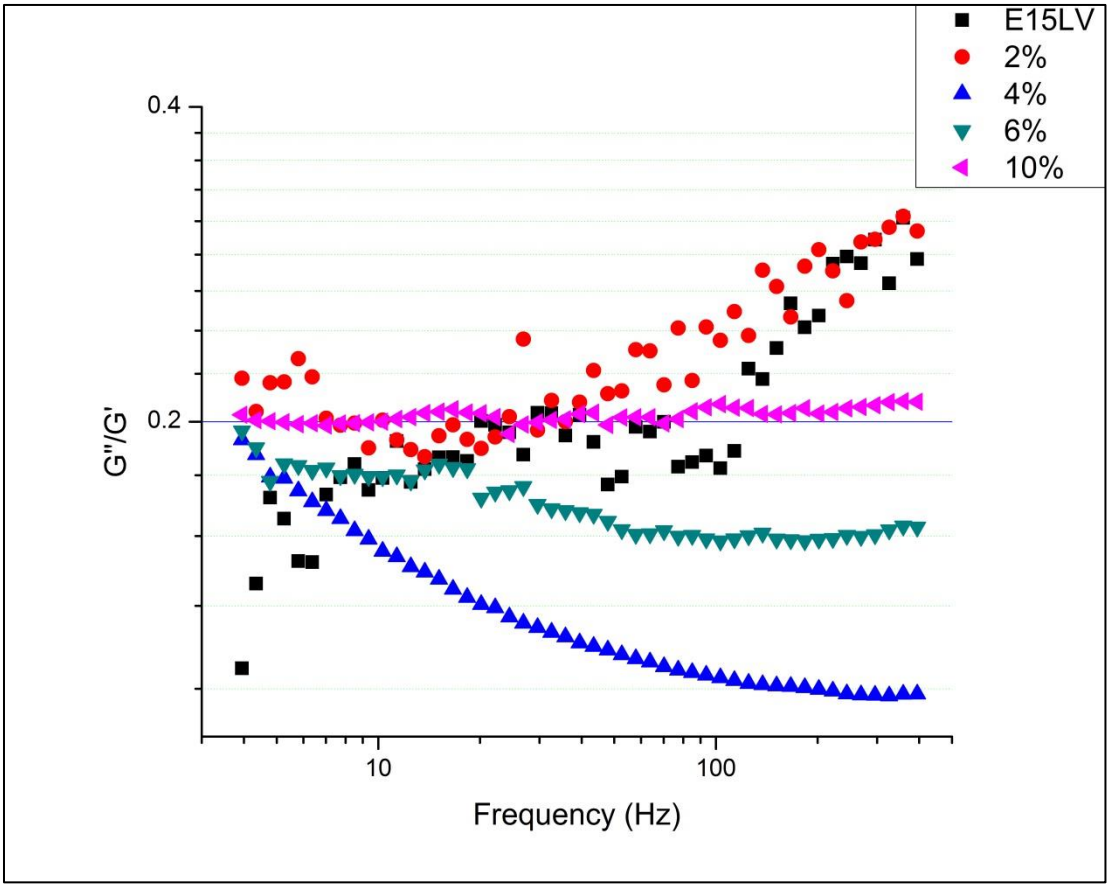


Figure 36. Viscoelastic behavior of HPMC E15LV – GF solutions @80 °C

3.4 Conclusions

Highly hydrophobic drugs like griseofulvin and fenofibrate in concentrations up to 5% (drug/polymer) were successfully suspended in different viscosity grade HPMC solutions. A significant effect on the gelation temperature was observed. T_{gel} decreases drastically when increasing the concentration of particles in the solution. This was attributed to

hydrophobic interaction between the particles and the polymer chains. This can be due to the higher affinity between HPMC and these drugs as proposed in the work of Romanski et al and Zhu et al, where the binding energy for both systems was estimated and correlated to the adsorption of the polymer in the drug surface.

A gel failure was observed when 7% by weight in solution of GF was suspended on 1% HPMC E4M. The gelation of the polymer initially was highly promoted by the presence of the particles, and T_{gel} was about the same as for 5% of GF. However, when the process surpasses T_{gel}, a failure in the gel structure occurs and the particles precipitate. The weight of the particles is larger than the strength of the hydrophobic interactions of the gel network that keeps them suspended.

Differences in the molecular weight of HPMC polymer were related to the ability of the polymer to form bridges between drug particles. The polymer molecular weight is a limiting factor in the gelation process, since there is a competition between polymer absorption on the surface of the drug and polymer-polymer interactions. These interactions are highly dependent on the polymer chain length.

3.5 References

1. Amidon G., Lennernäs H., Shah V., Crison J. A Theoretical Basis For A Biopharmaceutic Drug Classification: The Correlation Of In Vitro Drug Product Dissolution And In Vivo Bioavailability. Pharmaceutical Research 12(3) (1995); 413 – 420.
2. Acharya C., Seo P., Polli J., Mackerell A. Computational Model For Predicting Chemical Substituent Effects On Passive Drug Permeability Across Parallel Artificial Membranes. Molecular Pharmaceutics 5(5) (2008); 818 – 828.
3. Krishnaiah Y. Pharmaceutical Technologies For Enhancing Oral Bioavailability Of Poorly Soluble Drugs. Journal Of Bioequivalence & Bioavailability 2(2) (2010); 28 – 36.
4. Nagabandi V., Ramarao T., Jayaveera K. LIQUISOLID Compacts: A Novel Approach To Enhance Bioavailability Of Poorly Soluble Drugs. International Journal Of Pharmacy And Biological Sciences 1(3) (2011); 89 – 102.
5. Hammond R., Pencheva K., Roberts K., Auffret T. Quantifying Solubility Enhancement Due To Particle Size Reduction And Crystal Habit Modification: Case Study Of Acetyl Salicylic Acid. Journal Of Pharmaceutical Sciences 96(8) (2007); 1967 – 1973.
6. Sun J., Wang F., Sui Y., She Z., Zhai W., Wang C., Deng Y. Effect Of Particle Size On Solubility, Dissolution Rate, And Oral Bioavailability: Evaluation Using Coenzyme Q_{10} As Naked Nanocrystals. International Journal Of Nanomedicine 7 (2012); 5733 – 5744.
7. Liversidge G., Cundy K. Particle Size Reduction For Improvement Of Oral Bioavailability Of Hydrophobic Drugs: I. Absolute Oral Bioavailability Of Nanocrystalline Danazol In Beagle Dogs. International Journal Of Pharmaceutics 125 (1995); 91 – 97.
8. Van Eerdenbrugh B., Vermant J., Martens J., Froyen L., Van Humbeeck J., Augustijns P., Van Den Mooter G. A Screening Study Of Surface Stabilization During The Production Of Drug Nanocrystals. Journal Of Pharmaceutical Sciences 98(6) (2009); 2091 – 2103.
9. Rogers T., Gillespie I., Hitt J., Fransen K., Crowl C., Tucker C., Kupperblatt G., Becker J., Wilson D., Todd C., Broomall C., Evans J., Elder E. Development And Characterization Of A Scalable Controlled Precipitation Process To Enhance The Dissolution Of Poorly Water-Soluble Drugs. Pharmaceutical Research 21(11) (2004); 2048 – 2057.
10. Al-Ghazawi A., Nuguru K., Gianbattisto D. Immediate Release Tablet Containing Naproxen Sodium. U.S. Patent US 2003/0211150 A1. (2003)
11. Sing N., Zhang S., Liu C. Overview Of Factors Affecting Oral Drug Absorption. Asian Journal Of Drug Metabolism And Pharmacokinetics 4(3) (2004); 167 – 176.
12. Sievens-Figueroa L., Bhakay A., Jerez-Rozo J., Pandya N., Romañach R., Michniak-Kohn B., Iqbal Z., Bilgili E., Davé R. Preparation And Characterization

Of Hydroxypropyl Methyl Cellulose Films Containing Stable BCS Class II Drug Nanoparticles For Pharmaceutical Applications. International Journal Of Pharmaceutics 423 (2012); 469 – 508.
13. Acevedo A., Takhistov P., Pinzón De La Rosa C., Florian V. Thermal Gelation Of Aqueous Hydroxypropylmethylcellulose Solutionswith SDS And Hydrophobic Drug Particles. Carbohydrate Polymers 102 (2014); 74 – 79.
14. DOW Methoxel Cellulose Esters: Technical Handbook. Form No. 192-01062-0902 AMS (2002) USA.
15. Romanski F. The Production And Stabilization Of Pharmaceutical Nanosuspensions. Doctoral Dissertation Rutgers University. (2011)
16. Zhu W., Romanski F., Meng X., Mitra S., Tomassone M. Atomistic Simulation Study Of Surfactant And Polymer Interactions On The Surface Of A Fenofibrate Crystal. European Journal Of Pharmaceutical Sciences 42 (2011); 452 – 461.
17. Flory P. Principles Of Polymer Chemistry. Cornell University Press (1953), Ithaca, New York.
18. Viriden A., Wittgren B., Larsson A. Investigation of Critical Polymer Properties for Polymer Release and Swelling of HPMC Matrix Tablets. European Journal of Pharmaceutical Sciences 36 (2009); 297 – 309.
19. Hao T., Riman R. Calculation of Interparticle Spacing in Colloidal Systems. Journal of Colloid and Interface Science 297 (2006); 374 – 377.

4. PLASTICIZER EFFECT ON GELATION OF HPMC SOLUTIONS FOR DRUG DELIVERY APPLICATIONS

4.1 Introduction

Thin polymer films for drug delivery applications have been proposed as an advanced alternative to traditional tablets, capsules and liquids often associated with prescription and over the counter (OTC) medications [1-3], especially in applications where a more comfortable presentation is required. These films, mostly designed for oral administration, are designed to bypass the first metabolism path making the drug more bioavailable.[4, 5]

Most thin films formulations are composed of a biopolymer based matrix, active ingredient, coloring, flavoring and saliva stimulating agents, preservatives, and plasticizers.[6] The last mainly incorporated to improve the flexibility of the strip and reduce its brittleness.[7] Plasticizers also are used as viscosity modifiers in polymer solutions.

One of the most known and successful examples a dissolvable thin biopolymer have been applied is *Listerine Pocketpaks® oral care strips* [8], a Johnson & Johnson brand available in the market since 2007. A biopolymer matrix based on pullulan was used to suspend essential oils, such as menthol, thymol, methyl salicylate, and eucalyptol, which have an antiseptic effect. Xanthan gum, another polysaccharide, is used as a thickening

agent. This product takes advantage of the pullulan ability to suspend these oils in the films structure and its easy dissolution in humid environment.

Other systems have been proposed to the fabrication of edible (and transdermal) thin films for food and pharmaceutical applications [9-11], to administer drugs or nutraceutical ingredients via absorption. This drug delivery method has generated great interest for its convenience with pediatric and geriatric patients [12, 13] and for the development of "on the go" products and food coatings.

Plasticizers in film formulations have many applications related to the final desired mechanical properties of the films. Plasticizers may increase the flexibility, mechanical strength or durability of films, but at high concentration may affect the water vapor permeability (WVP) of the films. Also help to protect the active ingredient from oxidation. Many plasticizer compounds promote (or obstruct) gelation depending the type of plasticizer/polymer system, and might even promote interactions between polymer and drug particles, facilitating their stabilization. [14, 15] Plasticizers also have an important role in the water vapor permeability of the films since are able to reduce the hydrophobic interactions that provide the water transport barrier.[16]

Two types of plasticization can occur, internal and external. Internal plasticizers chemically modify a polymer chain through the addition of substituent groups attached via covalent bonds.[17] Most common plasticizers used in edible films and coating are

polyols, which include glycerol, sorbitol, PEG, sucrose, and propylene glycol. These plasticizers are able to perform an external plasticization, where they solvate and lubricate the polymer chains, lowering their glass transition temperature and also increasing the free volume.[18] The effectiveness of a plasticizer is dependent upon three aspects: size, shape, and compatibility with the polymer matrix.[19] The state of the plasticizer under normal storage conditions may also affect its permeability and flexibility. Solid plasticizers may have an "antiplasticizing" effect, decreasing matrix flexibility, while improving the vapor water permeability barrier.[20]

For drug delivery formulations the flexibility, strength, and swelling capabilities of the films are very important to ensure long term stability and suitable release of the active ingredient [21], many plasticizers are able to improve these properties. However, the perfect amount of plasticizer must be evaluated to avoid mechanical flaws in the film structure due to phase separation effects.

Phong and Takhistov have reported an optimal glycerol concentration range from 9 to 29% (by weight) to plasticize E15LV viscosity grade HPMC films [22], where glycerol enhances the flexibility of the polymer to improve the mechanical properties of the films. In their work, HPMC E15LV films were prepared by dry casting method and evaluated using optical microscopy to determine if there was a phase separation within the films. Other works attribute phase separation between plasticizer and polymer to and excess of plasticizer or incompatibility between them.[23, 24]

In the first section of this chapter, the effect of the concentration and type of plasticizer (Glycerol, D-sorbitol and PEG 400), on HPMC grade E4M was evaluated. Linear regression analyses were proposed to predict T_{gel} in HPMC solutions in presence of the evaluated plasticizers.

HPMC solutions have been reported to have a viscoplastic Bingham behavior.[25, 26] However, at high concentrations of glycerol a phase separation may occur.[27] In the second part of this chapter, steady-state rheological measurements are proposed to be used as a screening tool for phase separation prediction in HPMC-glycerol solutions.

Finally, HPMC-glycerol films were prepared by dry casting method to confirm the rheological results. Films were evaluated by NIR-CI (chemical imaging) to determine glycerol distribution through the films.

4.2 Experimental Section

4.2.1 Materials

Hydroxypropyl methylcellulose viscosity grade E4M and E15LV (CAS-No: 9004-65-3), glycerol >99.9% (CAS-No: 58-81-5), d-sorbitol (CAS-No: 50-70--4) and PEG Mn 400 (CAS-No: 25322-68-3), all were purchased from Sigma-Aldrich. All plasticizers were used without further modification. Deionized water was used as solvent for all solutions.

4.2.2 Sample preparation & Characterization

For the first experiments, stock HPMC E4M 2% solutions were prepared by heating deionized water to 80 °C while stirring, and adding the polymer by small quantities at a time until complete dissolution. After the entire polymer is incorporated, the temperature was reduced to 25 °C and the stirring was maintained from 24 to 36 hours. For HPMC-plasticizer solution preparation, the stock HPMC solution was diluted to 1% (by weight) and the desired amount of plasticizer (1, 2, 5, 7, 8, 9 and 10% by weight) were added. Solutions were kept under constant stirring for an additional 8 to 12 hours to ensure complete dissolution and homogenization. Plasticizers used (Figure 37) were chosen because their small size and water solubility.

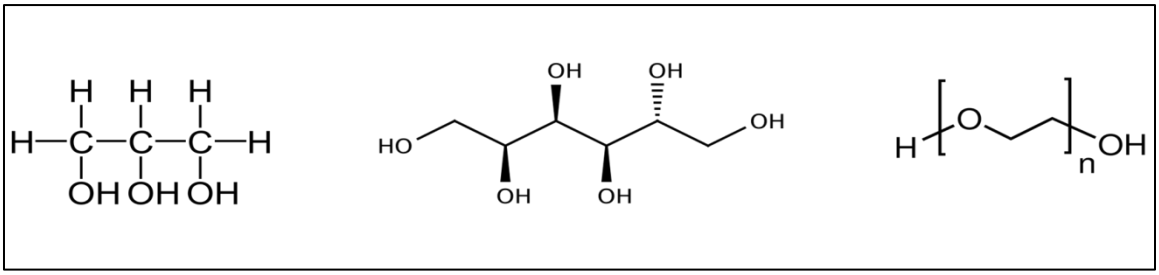


Figure 37. Glyceril, sorbitol and PEG molecular structure

All samples were subjected to oscillatory shear measurements to determinate the gelation temperature. A temperature ramp from 25 to 80 °C at a heating rate of 0.5 °C/min was applied. All samples were transferred to the rheometer cell and let to rest for at least 20 minutes to stabilize any remaining stresses from the loading.

For the second section, stock HPMC 8% E15LV viscosity grade solutions were following the same procedure previously described. For HPMC-glycerol solution preparation, the stock HPMC solutions were diluted to 2.5 and 5% (by weight) and the desired amount of plasticizer was added (up to 40% by weight). Solutions were kept under constant stirring for 12 hours to ensure complete dissolution and homogenization, before being used in the rheological experiments.

Steady-state rheological measurements were performed in an Anton Paar MCR 302 modular rheometer and double gap geometry using a shear rate ramp from 0.01 to 100 s^{-1} at a constant 25 °C temperature. All samples were transferred to the rheometer cell and left to rest for at least 20 minutes to stabilize any remaining stresses from the loading process. All measured were performed three times and the resulting data was analyzed by ANOVAs using Minitab, for statistical consistency.

HPMC-glycerol films were prepared pouring 30 grams of the polymer-plasticizer solution on a tempered glass plate and left to dry completely on a Thermo Precision 655 convection oven at 35 °C for 12 hours. After drying, the films were cut into one square inch sections and analyzed on a Malvern SyNIRgy NIR-CI, with an operating wavelength length of 1200 to 2400 nm, and a 40 μm/pixel magnification.

4.3 Results and discussion

4.3.1 Plasticizers effect on T_{gel} of HPMC solutions

In this section, HPMC viscosity grade E4M solutions with different concentrations of glycerol, d-sorbitol and PEG were evaluated. The plasticizer effect was evaluated in 1% HPMC solutions with plasticizer concentrations up to 10% by weight.

It has been reported that plasticizers improve the mobility of the polymer chains in the solution, which in theory will lubricate the polymer chains promoting the structures increasing the hydrophobic interaction between them by the exposition of the methoxyl groups.

From the rheological measurements a clear trend for glycerol and d-sorbitol to reduce the T_{gel} (coagulant effect) of the HPMC in the solution is observed (Figure 38). This effect is homologue to a salting-out effect by salt addition to the solvent [28], where by electrolyte-non electrolyte interactions some solutes as polymers or proteins precipitate (agglomerate) due to the increase of hydrophobic interactions. This phenomenon is attributed to the increase in hydrophobicity of the solvent induced by slightly polarizable ions with high surface charge density, which stimulates an increase in the hydrophobic interactions of macromolecules.[29]

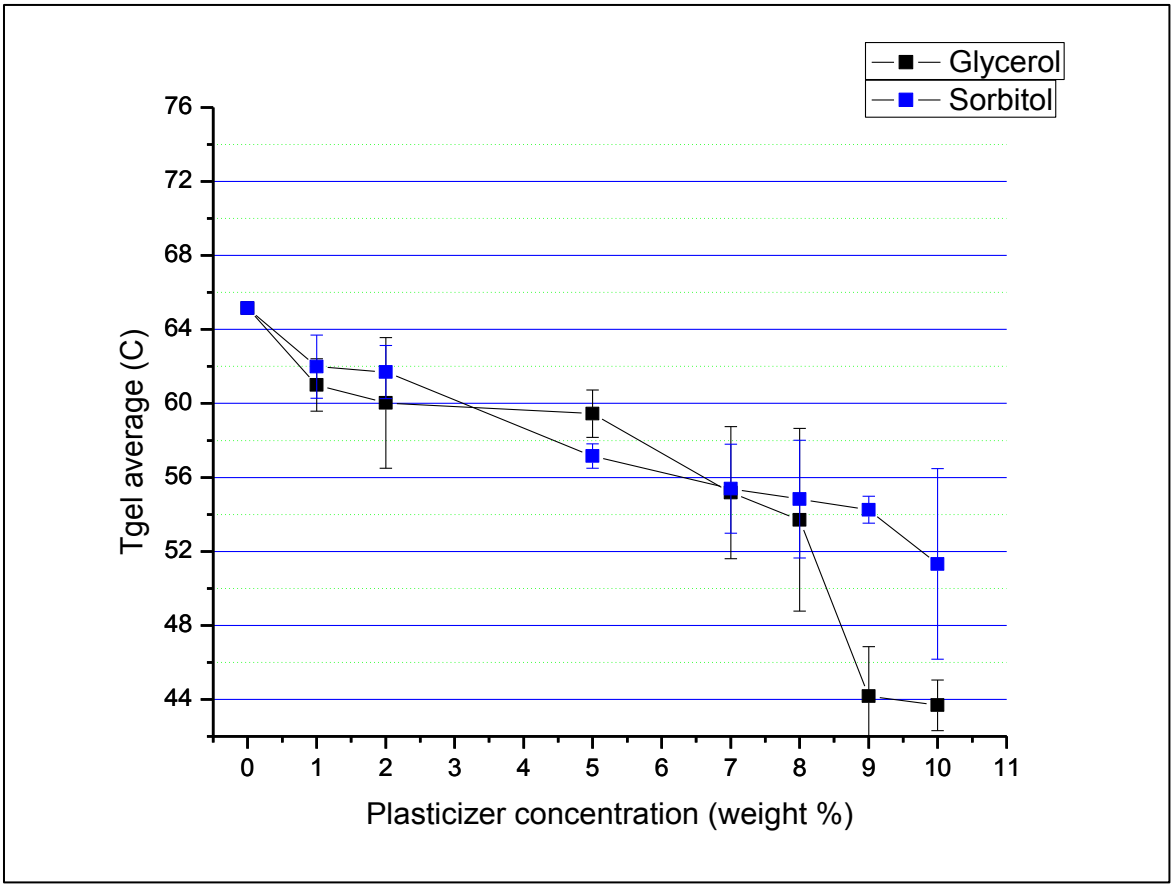


Figure 38. Effect of the addition of plasticizer in HPMC-d-Sorbitol, HPMC-glycerol solutions

Similar, mostly linear behavior was observed for glycerol and sorbitol in concentrations up to 8%, above that concentration glycerol exhibited a significant decrease in T_{gel} which is attributed to agglomeration of the polymer by a reduction in its solubility in the aqueous medium (water-glycerol mixture).

For PEG, an increase in T_{gel} was observed in concentrations up to 5%. This effect occurs because PEG has a solubilizing effect on HPMC polymer molecules in the solution. PEG promotes the solvation of HPMC molecules, preventing the disruption of the water cages around the hydrophobic sites in the polymer, which reduces the hydrophobic interactions between polymer molecules; this in turn increases the gelation temperature (energy needed to disrupt the water cages around the hydrophobic sites, which are in charge for the gelation). This effect is more noticeable in concentrations between 2 and 5%, small quantities of PEG have no significant effect on the gelation mechanism.

PEG molecules are relatively large (compared with glycerol and sorbitol), and at higher concentrations these molecules can agglomerate forcing the HPMC molecules to form bundles which promote the hydrophobic interaction, generating an inverse plasticizer effect reducing T_{gel}. The plasticizing effect of PEG in the studied concentration range can be observed on detail on the Figure 39.

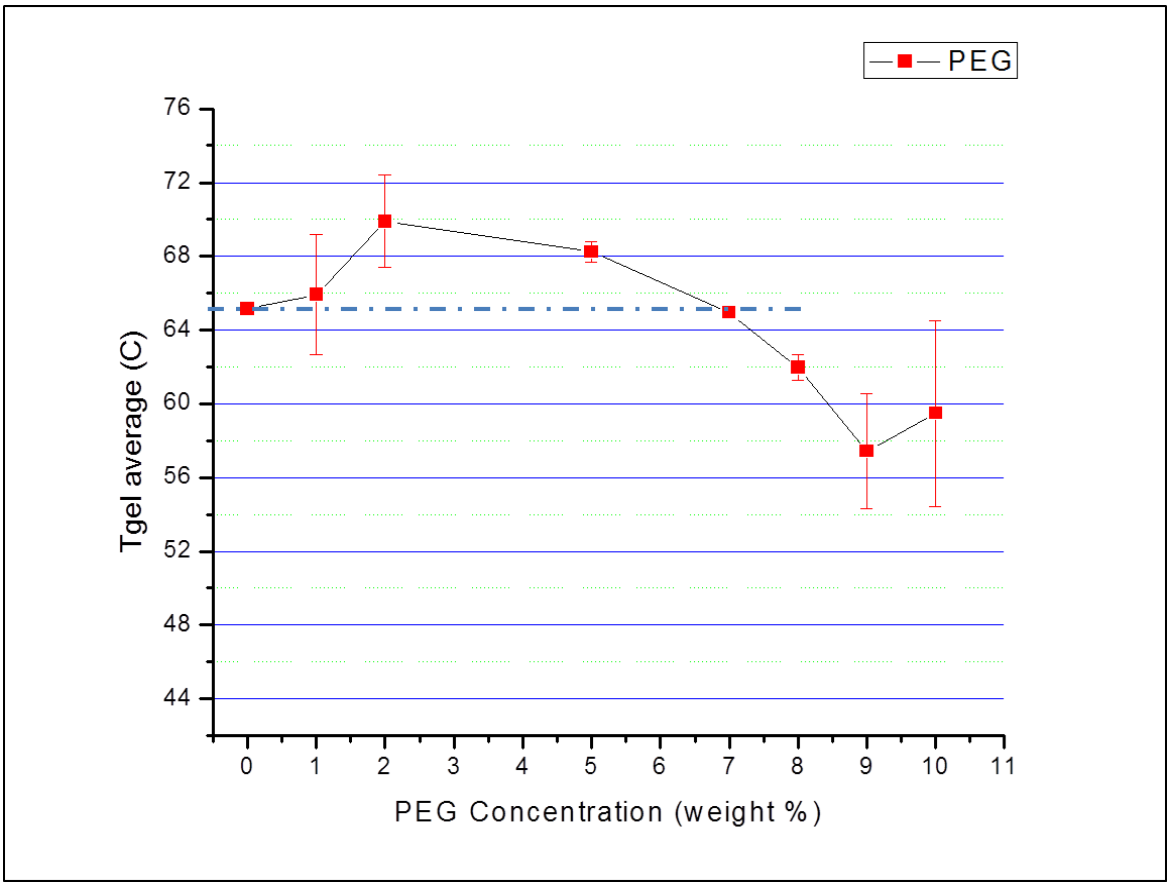


Figure 39. Effect of the addition of PEG in Tgel of HPMC solutions

From the linear regression analysis it can be concluded that the type of plasticizer and its concentration, both have a significant effect on T_{gel}, p-values below 0.05 for both factors were obtained; more details can be found on the appendix.

Figure 40 summarizes the T_{gel} data distribution for all plasticizers. Glycerol exhibits the largest variation in T_{gel}; this is attributed to the big changes in T_{gel} at higher concentrations due to the polymer agglomeration caused by an initial phase separation stage.

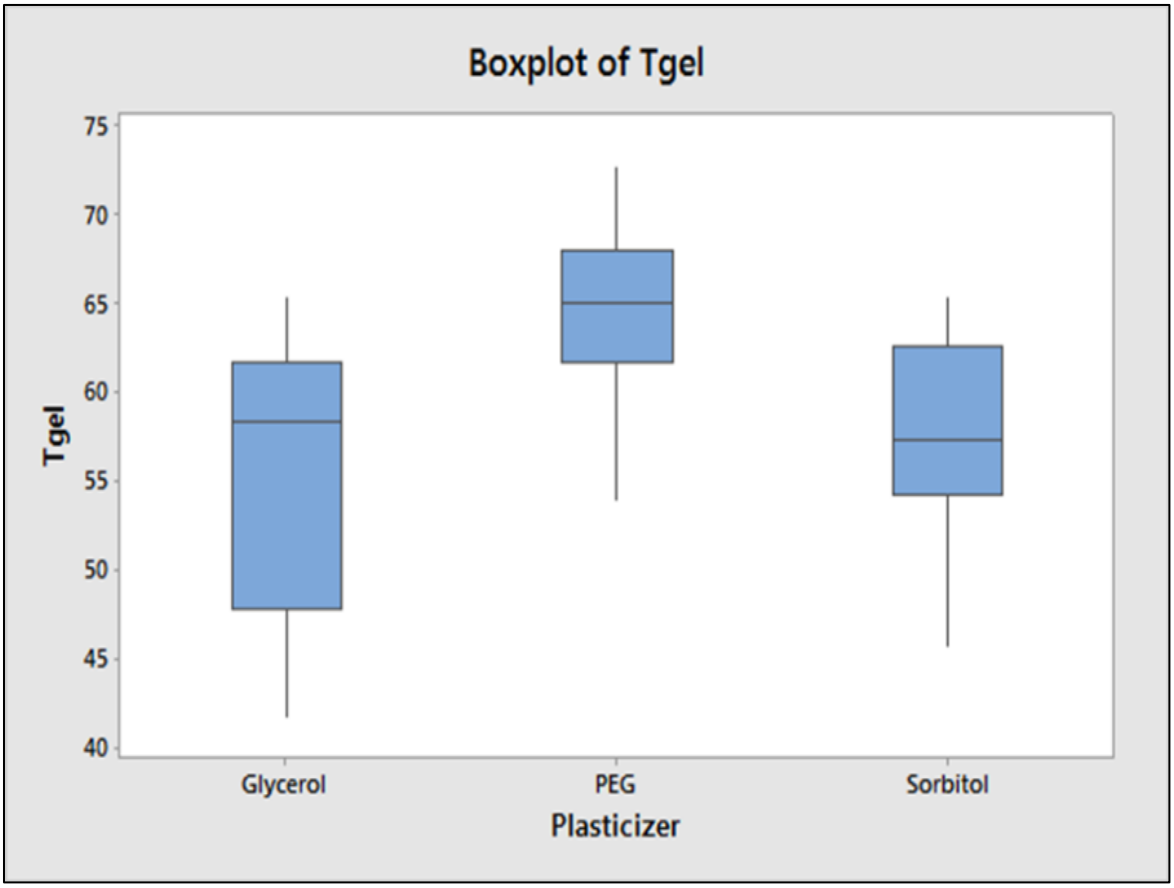


Figure 40. Data distribution/variability for glycerol, PEG, sorbitol in HPMC 1% solutions

Based on the linear regression analysis, three linear approximations were obtained to predict the gelation temperature of the evaluated systems,

$$Glycerol, \quad T_{gel} = 62.123 - 1.300 * C$$

$$PEG, \quad T_{gel} = 70.956 - 1.300 * C$$

$$Sorbitol, \quad T_{gel} = 64.549 - 1.300 * C$$

where *C* represents the concentration of the plasticizer. These equations represent the behavior of the systems for plasticizer concentrations of 1% (by weight) an above.

Glycerol exhibits a very interesting behavior. While T_{gel} displays a linear dependence with the sorbitol and glycerol in concentrations up to 8%, at higher concentrations of glycerol a discontinuity in the linearity is observed. This behavior is attributed to an initial stage of glycerol clusters' formation where the glycerol molecules agglomerate. In the second part of this chapter a more detailed evaluation of this behavior is presented.

4.3.1 Rheology as screening tool for film formulations

The objectives of this section was to determine the effect of glycerol in the rheology of HPMC solutions and determine if there is a correlation between solution rheology and the morphological properties of dry-casted films, such that it may be used as a screening tool for film formulations. For this purposes steady-state rheological measurements were performed on 2.5 and 5% HPMC solutions with glycerol concentrations up to 40% by weight.

From the steady-state rheological measurements a shear thinning followed by a Newtonian plateau was observed for all HPMC solutions. However, at concentrations above 30% (by weight) the constant viscosity (Newtonian plateau) zone disappears. This behavior

suggests a change in the morphology such as the presence of plasticizer rich zones which may generate discontinuities in the viscosity curve.

In Figures 41 to 44 the effect of glycerol concentration in the viscoplastic behavior of the HPMC 2.5 and 5% solutions is shown. The results were divided in two graphs for each HPMC concentration, since the viscosity curves for 20 to 40% glycerol are in different order of magnitude and the effect on the lower concentrations is not appreciated.

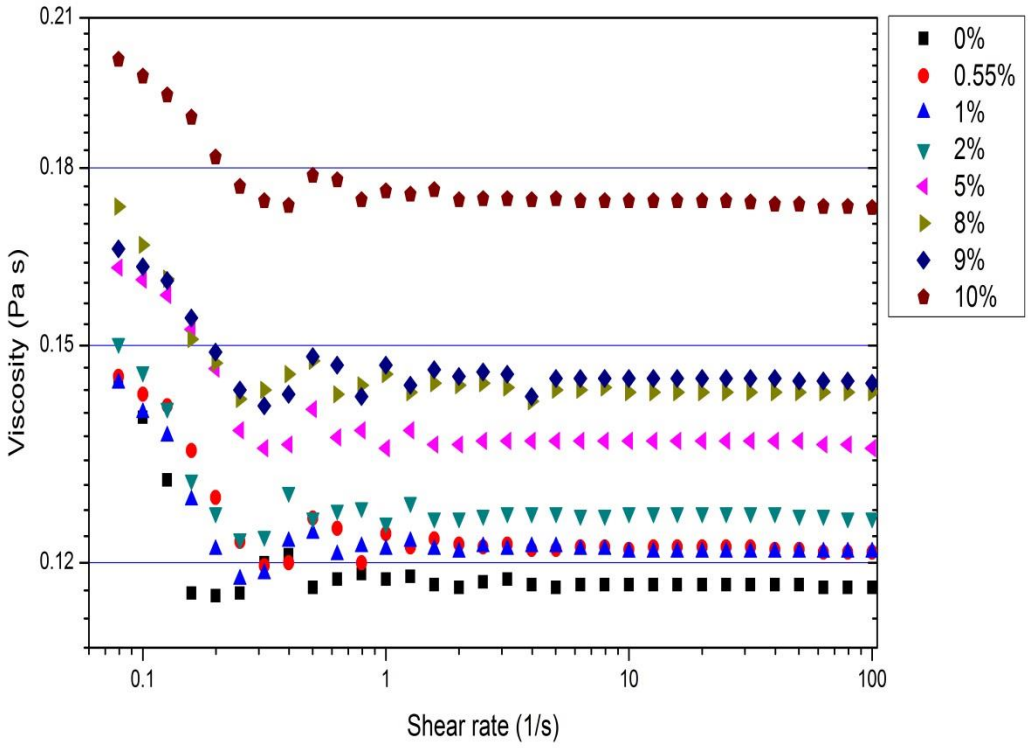


Figure 41. Effect of the glycerol concentration in 5% HPMC E15LV solutions for glycerol concentrations up to 10%

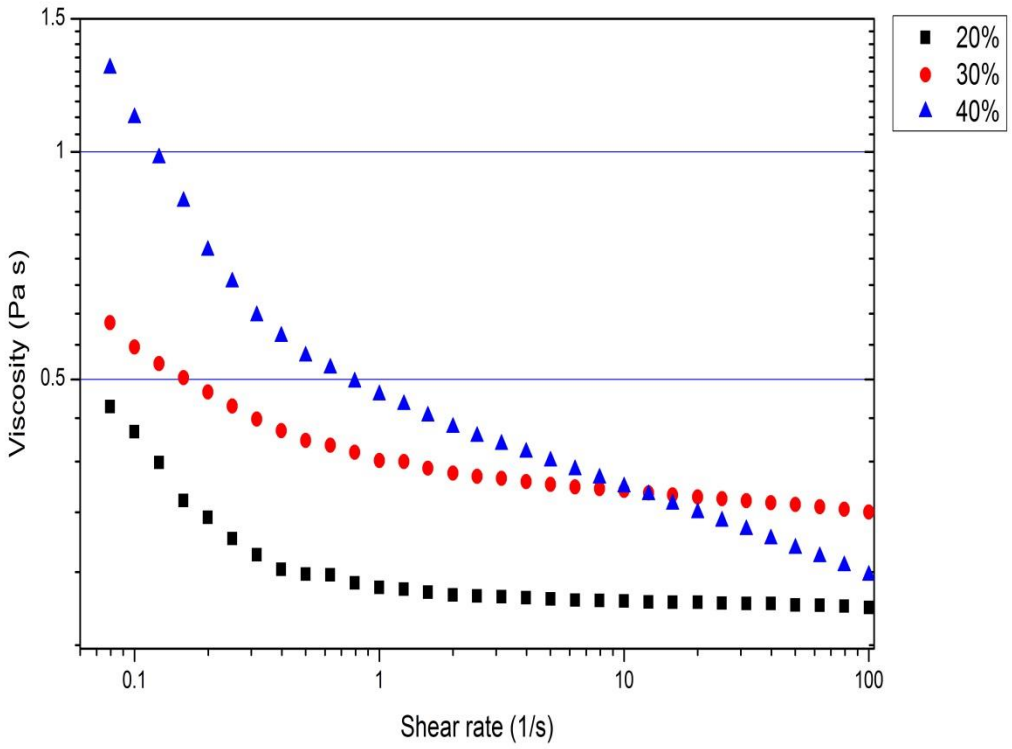


Figure 42. Effect of the glycerol concentration in 5% HPMC E15LV solutions for glycerol concentrations 20 to 40%

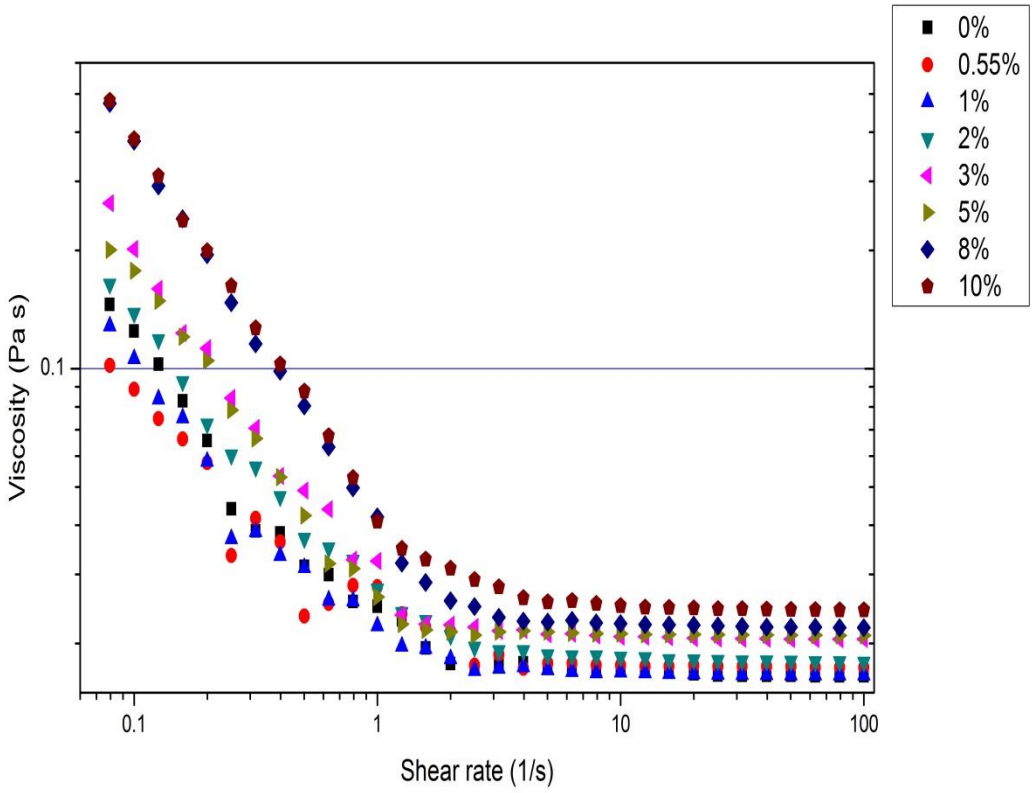


Figure 43. Effect of the glycerol concentration in 2.5% HPMC E15LV solutions. Glycerol concentrations up to 10%

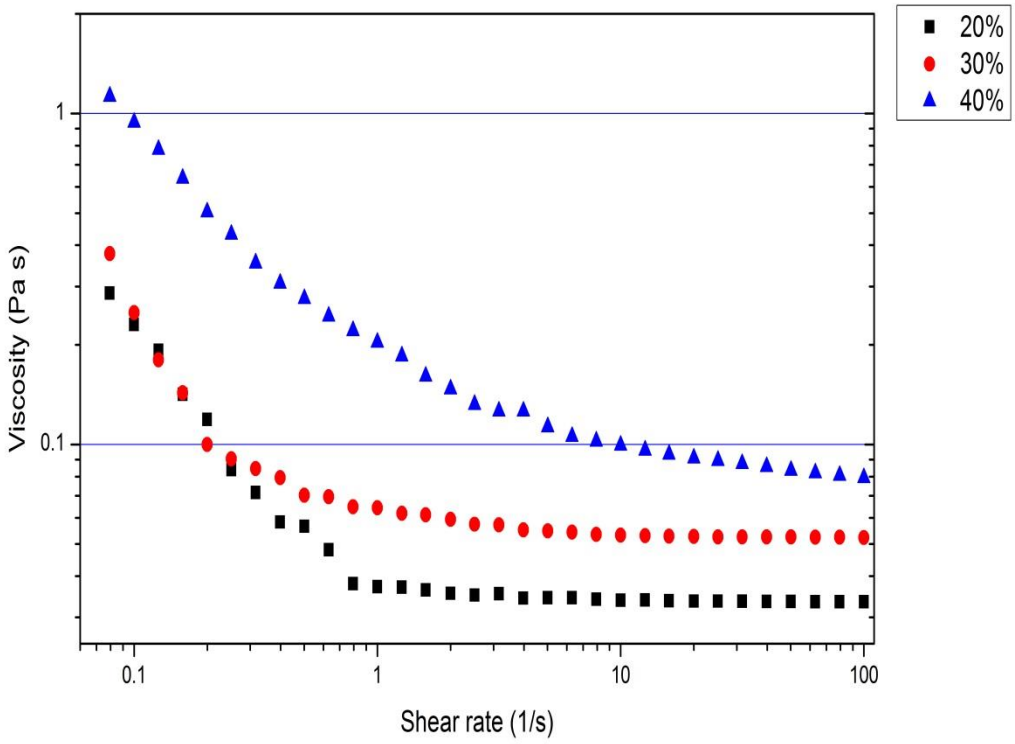


Figure 44. Effect of the glycerol concentration in 5% HPMC E15LV solutions for glycerol concentrations 20 to 40%

It can be observed that all samples behave as a Bingham plastic, where an initial stress (yield stress) has to be applied to make the polymer flow. As the shear rate increases the viscosity decreases until reaches a constant value. However, for both HPMC solutions at glycerol concentrations above 30% the plateau disappears (Figures 42 and 44).

From the Bingham model, calculation from the yield stress can be made by fitting models to the measured rheograms and extrapolating to zero shear rate. The Yield stress is obtained by the intercept on y of the curve.

$$Bingham\ viscosity\ model: \sigma = \sigma_0 + \eta_B \dot{\gamma}$$

Where σ is the stress, σ_0 is the yield stress, η_B is the Bingham viscosity or plastic viscosity and $\dot{\gamma}$ is the shear rate.

Glycerol in solution have been reported to behave in three different ways, depending on the concentration on the concentration and affinity with the solvent and polymer [30], glycerol can (1) interact with the polymer and form bonds with the polymer chains (Figure 45a), (2) can act as a lubricant and stay in the solvent around polymer chains (Figure 45b) or (3) agglomerate in plasticizer rich zones between the polymer chains (Figure 45c).

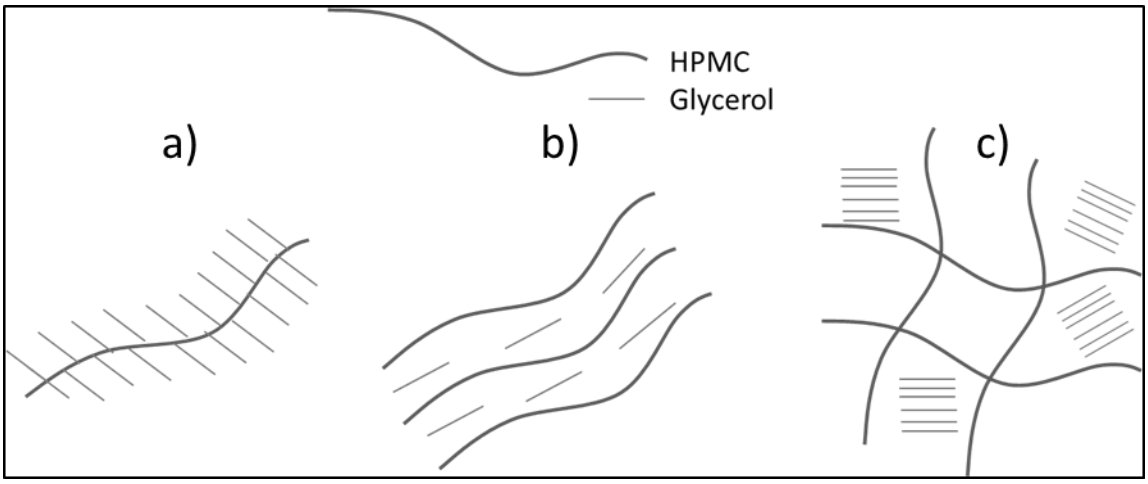


Figure 45. Glycerol-HPMC interactions in solution

At low glycerol concentrations (a) and (b) are the predominant arrangements. When the glycerol concentration increases, it have been reported that and agglomeration of plasticizer molecules can occur due to partial miscibility between the HPMC and glycerol (c).[27] As result, internal fissures may appear in the film.

Yield stresses and plateau viscosities were calculated using the Bingham viscosity model. Figures 46 and 47 illustrate the plateau viscosity behavior when increasing the glycerol concentration for 2.5 and 5% HPMC solutions. A constant increase viscosity for both HPMC concentrations is observed when increasing the glycerol concentration, this suggest and increases in the R_g (radius of gyration) of the polymer (stretching of the polymer molecules) due the plasticizer effect, this effect may cause an agglomerating effect of glycerol on the polymer chains.

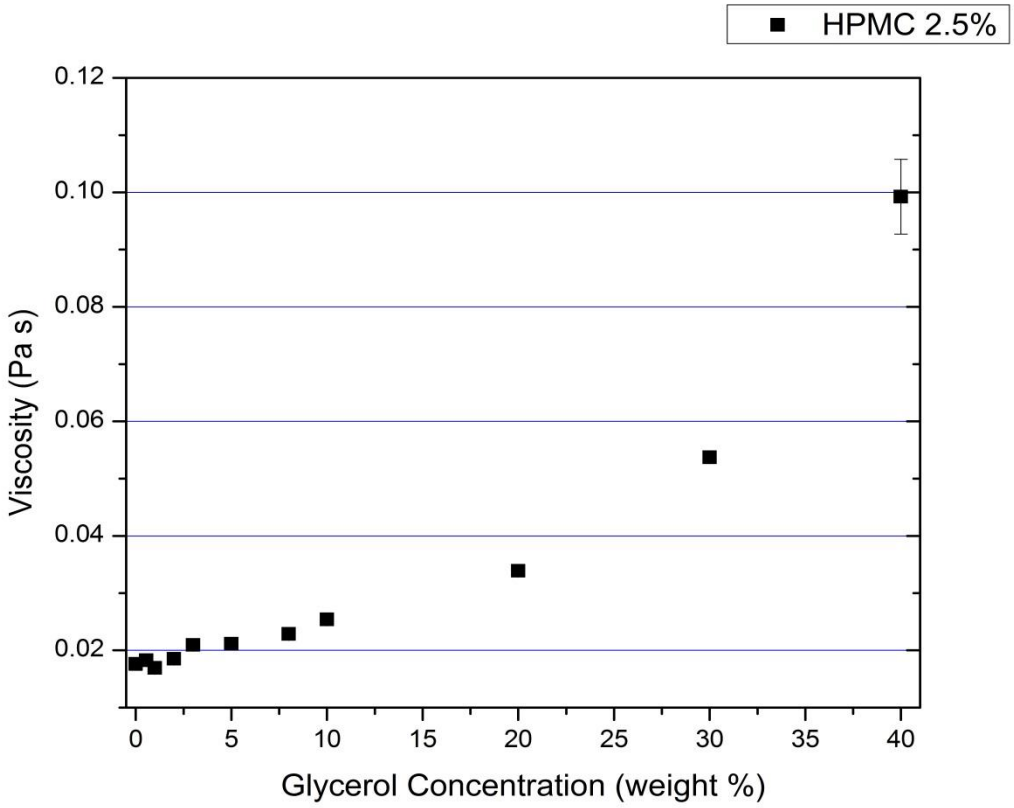


Figure 46. Plateau viscosity measurements for HPMC 2.5% - glycerol solutions

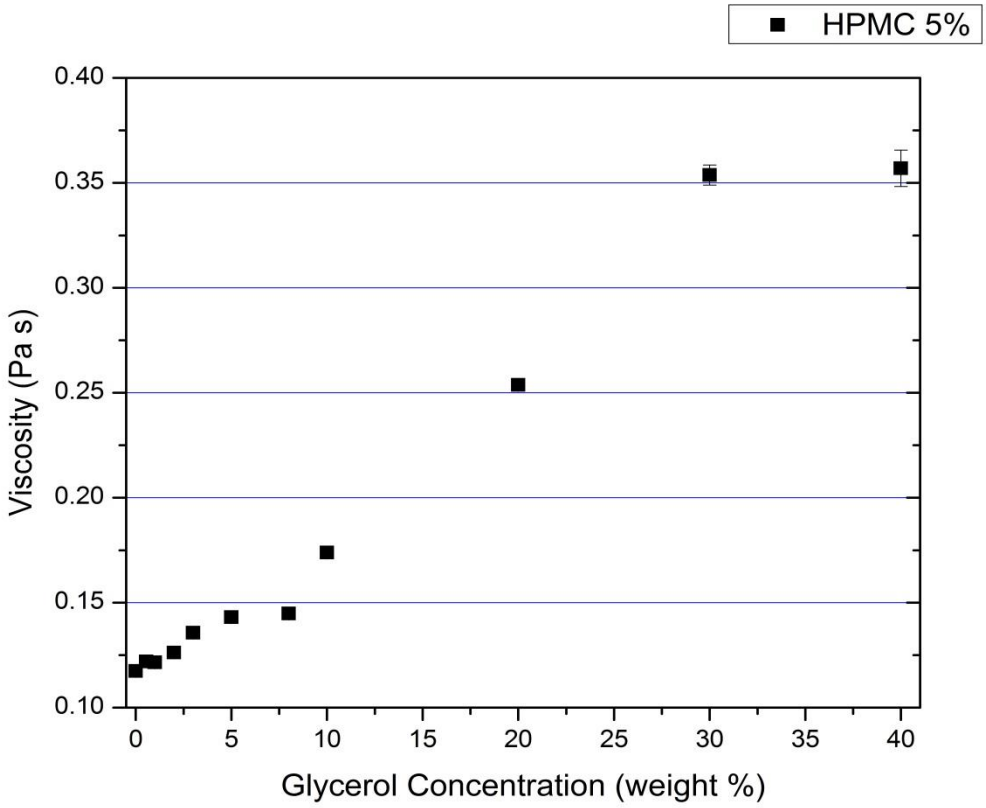


Figure 47. Plateau viscosity measurements for HPMC 5% - glycerol solutions

Figures 48 and 49 show how the yield stress is affected by the glycerol concentration. For both 2.5 and 5% HPMC solutions, an increase in the yield stress is observed when concentration of glycerol reaches 30%. This is attributed to the behavior shown on Figure 45c, where glycerol forms aggregates, creating discontinuities in the polymer solution which affects the flow properties of the sample.

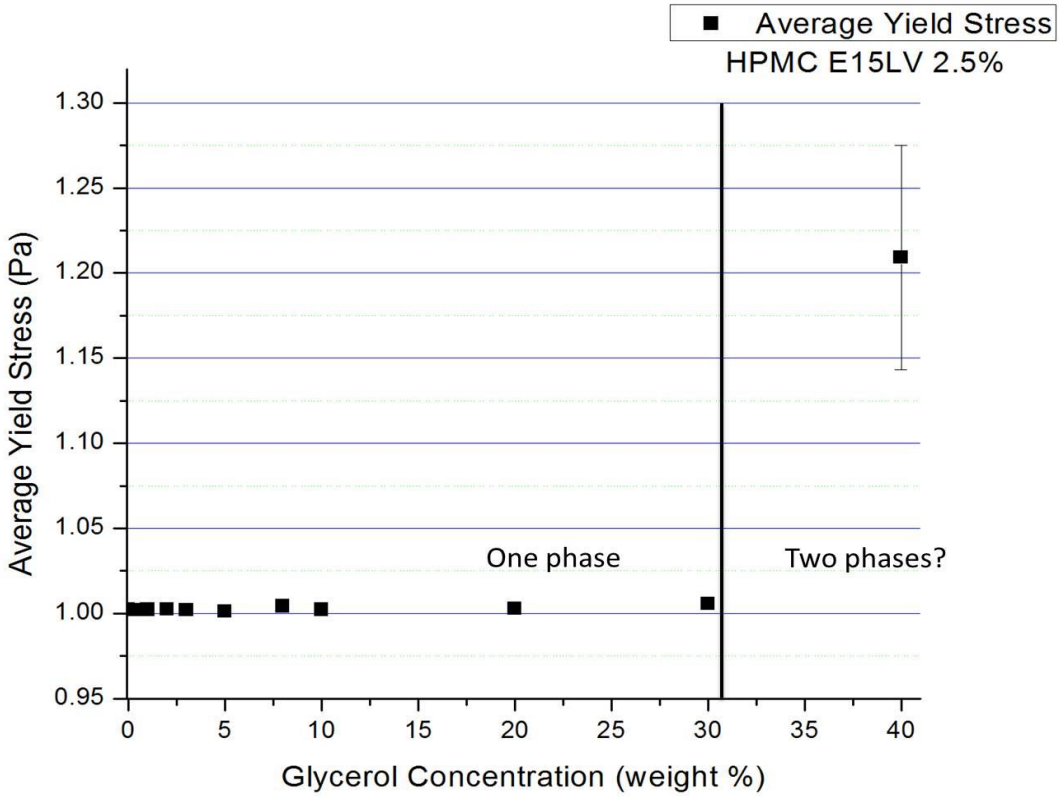


Figure 48. Effect of the glycerol concentration on the yield stress on 2.5% HPMC solutions

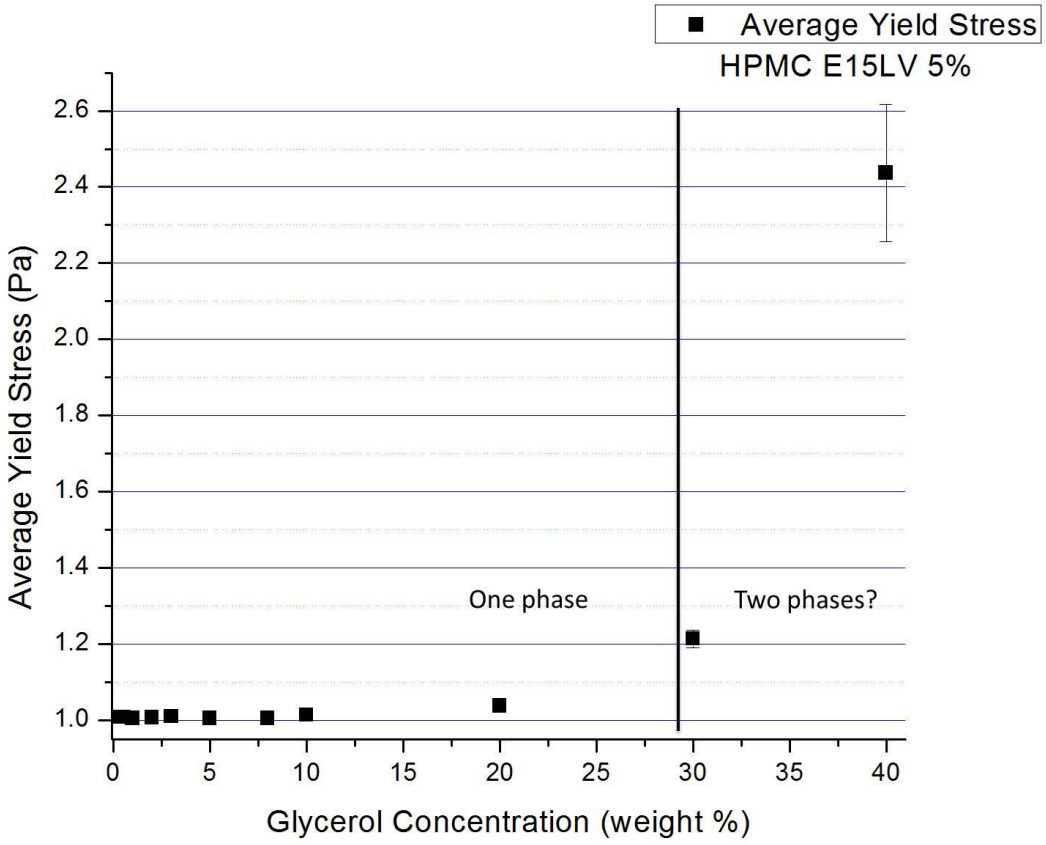


Figure 49. Effect of the glycerol concentration on the yield stress on 5% HPMC solutions

This increase in the 30 – 40% region is attributed to phase separation between the polymer and the plasticizer. At higher concentration of polymer, the effect is more noticeable due to the miscibility effect between glycerol and HPMC increases.

NIR-CI was used as a validation tool. Films of HPMC-glycerol were prepared by dry-casting method, using the same HPMC-glycerol concentrations. NIR spectrums of the

pure components were obtained previous of the films analysis to determine the glycerol distribution in the 0.55, 8 and 30% films. After getting the images and spectrums for all samples, an image analysis is conducted using ISys Chemical Imaging analysis software.

For the imaging analysis first the removal of the "back" and "dark" spectrum for all samples is required. The dark spectrum represents the noise generated by a black focus point where the light from the NIR lamps is reflected, and the back is the noise generated by a highly polished ceramic stones used to place the sample. Arithmetic operations between the sample spectrums and the back and dark spectrums are performed to remove the noise in the measurements.

After the removal of the dark and back spectrums from the images, a spectral "Triangle squared' Fourier filter is applied to the sample to optimize the curves and masking the remaining noise. After this optimization, the normalization and remotion of "bad pixels" in the images is done (see Figure 50), for this, ISys averages the information around a bad pixel in the sample with the information of the surrounding pixels to reduce the discontinuities in the film's image.

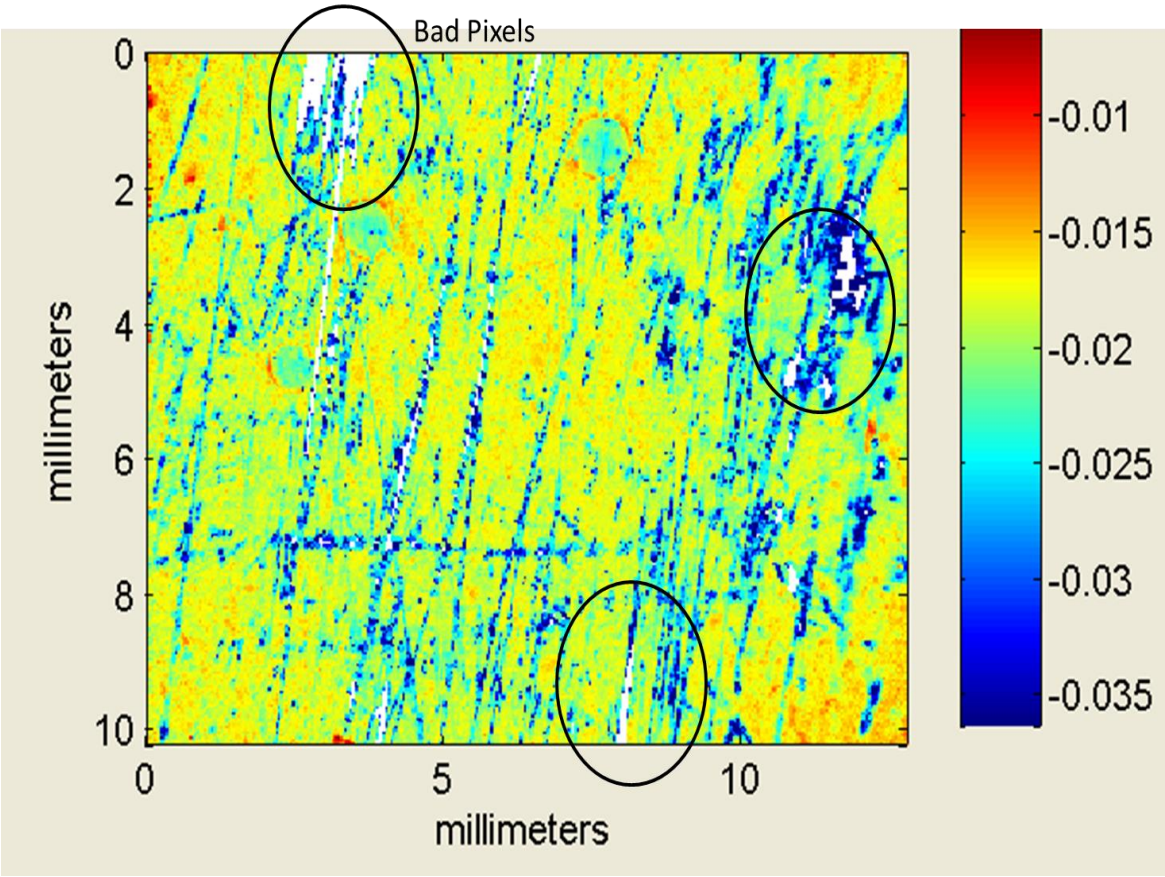


Figure 50. Bad pixels analysis in HPMC-Glycerol 30% untreated film NIR-CI Image

After these pretreatments all the data is transformed using a 2nd derivate modification filter. A spectral median filter replaces each point of the spectrum with the median of the values of a user-set neighborhood of that point. This is very effective in removing sharp spikes in a spectrum while preserving the overall shape of the waveform.

Finally, concatenations of all the spectrums (images) for a single glycerol concentration are combined in an only composite image (Figure 51). Concatenation is useful to link the data sets for different samples so that they are processed in exactly the same way. This function fuses data sets along the specified axis.

After concatenation, a statistical analysis is performed on the images, to eliminate all the pixels that are between a tolerance range (two times de standard deviation of the mean glycerol concentration for all films), the remaining pixels are considered to represent agglomeration or plasticize rich zones in the films. Binary (black and white) images are constructed with this information to provide more detailed representation of the plasticizer distribution on the films (Figure 52).

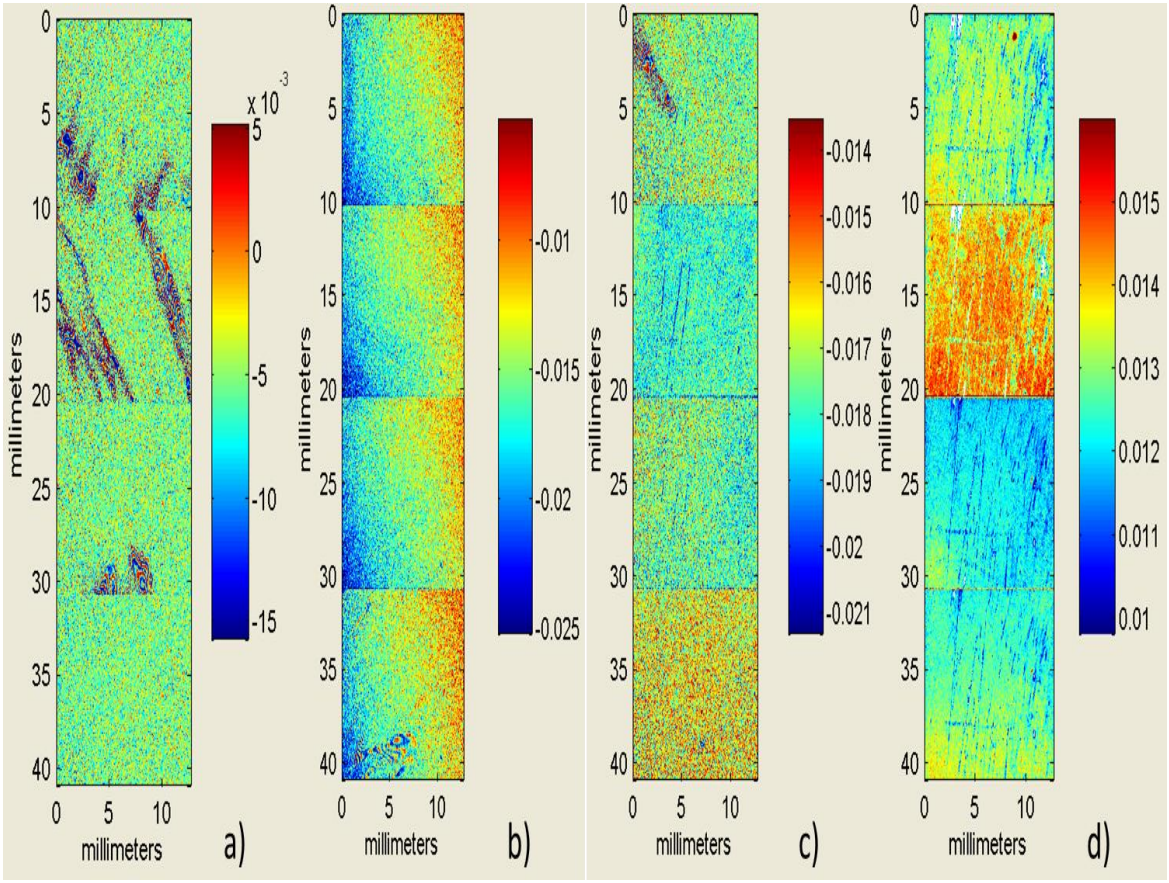


Figure 51. NIR-CI concatenated images for HPMC-glycerol films a) HPMC pure, b) 0.55% glycerol, c) 8% glycerol, d) 30% glycerol

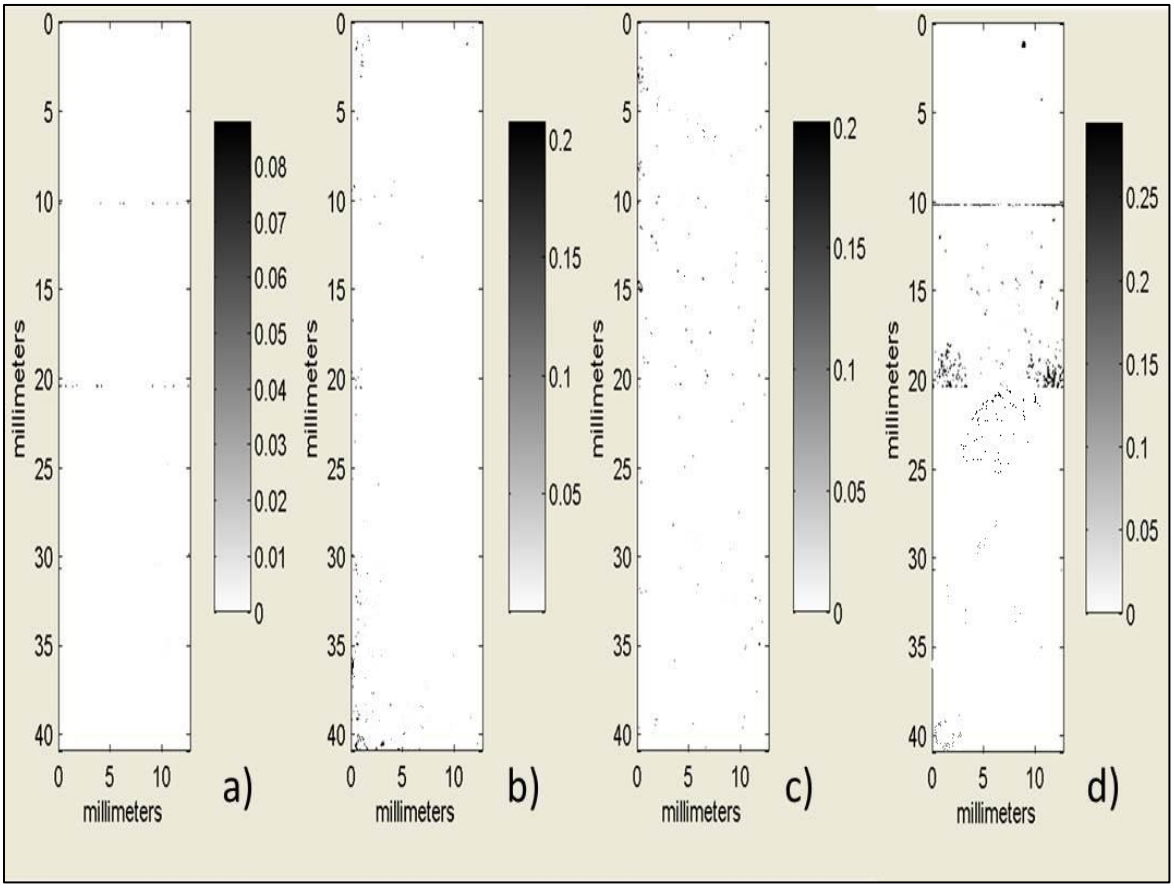


Figure 52. NIR-CI binary images for HPMC-glycerol films a) HPMC pure, b) 0.55% glycerol, c) 8% glycerol, d) 30% glycerol

When increasing the glycerol concentration, the amount and size of the black areas in the binary images increases. These areas represent plasticizer rich zones in the films caused by a phase separation phenomena between the polymer and plasticizer. Data validation methods and linear regression analysis is shown in detain on the appendix.

4.4 Conclusions

The plasticizing effect was observed in two different ways. A solubilizing effect of the HPMC molecules was observed with the addition of PEG in concentrations up to 5%. This behavior occurs because small PEG molecules may be attracted to the HPMC, acting as a solvent. These hydrophobic interactions block the polymer-polymer interactions that cause gelation, requiring more energy to achieve it. This behavior was reported previously by Laboulfie and collaborators in their work on the effect of different molecular weight PEGs on the mechanical resistance and thermal behavior of composite films for HPMC solutions.[31] At higher PEG concentrations, an opposing behavior is observed, and T_{gel} decreases when increasing the concentration of PEG in the solution, this can be attributed to the agglomeration of HPMC molecules caused by a phase separation with PEG.

The addition of glycerol and d-sorbitol to HPMC solutions originates a decrease in the gelation temperature of the samples. This behavior is attributed to a coagulant effect on the HPMC molecules by these small soluble molecules, causing agglomeration of the polymer molecules which increases the polymer-polymer interactions and promotes gelation. In some cases, the effect can be so strong that the polymer can precipitate after agglomeration.

The addition of plasticizer in film formulation for the enhancement of the mechanical properties can be related to the behavior of the precursor solutions. When the plasticizer

reduces T_{gel}, a more flexible yet fragile film is formed. Higher gelation temperatures may indicate better mechanical strength, but less malleability can be expected. This is the result of the organization of the polymer chains due to the presence of the plasticizer molecules.

The use of simple steady-state rheological measurements was successfully implemented as screening tool for film formulations. The morphological properties of dry-casted films were successfully predicted by relating the viscoelastic behavior of the films precursor solutions, applying a shear rate ramp at constant temperature.

Based on the results presented in the second section of this chapter, the use of in-line viscosity meters, or rheometers can be implemented as a process analytical technology (PAT) tool for controlling the morphological and mechanical properties of films in continuous industrial process. The quality of the product could be monitored in real-time based on a single viscosity measurement of the bulk solution.

From our rheological measurements and previous studies cited in this chapter, [takhistov 10], it can be expected that HPMC-glycerol films with glycerol concentrations between 9 and 30% have better mechanical properties. In this case, glycerol as plasticizer has a lubricant effect on HPMC films improving the polymer chain stretching and enhancing the viscosity of the samples below 29%.

The use of spectroscopic characterization methods like NIR-CI has been demonstrated to be a confident validation method for dry-casted films. In pharmaceutical films applications or food coating continuous manufacturing could be implemented as an excellent quality control tool.

4.5 References

1. Pepas N., Keys K., Torres-Lugo M., Lowman A. Poly(Ethylene Glycol)-Containing Hydrogels In Drug Delivery. Journal Of Controlled Release 62 (1999); 81 – 87.
2. Vartiainen J., Vähä-Nissi M., Harlin A. Biopolymer Films And Coating Packaging Applications – A Review Of Recent Development. Materials Sciences And Applications 5 (2014); 708 – 718.
3. Tang C., Guan Y., Yao S., Zhu Z. Preparation Of Ibuprofen-Loaded Chitosan Films For Oral Mucosal Drug Delivery Using Supercritical Solution Impregnation. International Journal Of Pharmaceutics 473 (2014); 434 – 441.
4. Xiao L., Yi T., Liu Y. A New Self-Microemulsifying Mouth Dissolving Film To Improve The Oral Bioavailability Of Poorly Water Soluble Drugs. Drug Development And Industrial Pharmacy 39(9) (2013); 1284 – 1290.
5. Li X., Zhang R., Liang R., Liu W., Wang C., Su Z., Sun F., Li Y. Preparation And Characterization Of Sustained-Release Rotigotine Film-Forming Gel. International Journal Of Pharmaceutics 460 (2014); 273 – 279.
6. Leung S., Leone R., Kumar L. Fast Dissolving Orally Consumable Films. U.S. Patent US 6596298 B2. (2003)
7. Fadda H., Hernandez M., Margetson D., Mcallister M., Basit A., Brocchini S., Suarez N. The Molecular Interactions That Influence The Plasticizer Dependent Dissolution Of Acrylic Polymer Films. Journal Of Pharmaceutical Sciences 97(9) (2008); 3957 – 3971.
8. Product Website: Https://Www.Listerine.Com/Products/Listerine-Go/Listerine-Pocketpaks-Oral-Care-Strips
9. François N., Daraio M. Preparation And Characterization Of Scleroglucan Drug Delivery Films: The Effect Of Freeze-Thaw Cycling. Journal Of Applied Polymer Science 112 (2009); 1994 – 2000.
10. Ham A., Rohan L., Boczar A., Yang L., Buckheit K., Buckheit R. Vaginal Film Drug Delivery Of The Pyrimidinedione IQP-0528 For The Prevention Of HIV Infection. Pharmaceutical Research 29 (2012); 1897 – 1907.
11. Moreira R., Teixeira J., Furuyama-Lima A., De Souza N., Siqueira A. Preparation, Characterization And Evaluation Of Drug-Delivery Systems: Pectin And Mefenamic Acid Films. Thermochimica Acta 590 (2014); 100 – 106.
12. Buck M. Alternative Forms Of Oral Drug Delivery For Pediatric Patients. Pediatric Pharmacotherapy 19(3) (2013). University Of Virginia – Children's Hospital.

13. Ghodake P., Karande K., Osmani R., Bhosale R., Harkare B., Kale B. Mouth Dissolving Films: Innovative Vehicle For Oral Drug Delivery. International Journal Of Pharma Research & Review 2(10) (2013); 41 – 47.
14. Vaithiyalingam S., Nutan M., Reddy I., Khan M. Preparation And Characterization Of A Customized Cellulose Acetate Butyrate Dispersion For Controlled Drug Delivery. Journal Of Pharmaceutical Sciences 91(6) (2002); 1512 – 1522.
15. Zhu Y., Mehta K., Mcginity J. Influence Of Plasticizer Level On The Drug Release From Sustained Release Film Coated And Hot-Melt Extruded Dosage Forms. Pharmaceutical Development And Technology 11 (2006); 285 – 294.
16. Tanaka M., Iwata K., Sanguandeekul R., Handa A., Ishizaki S. Influence Of Plasticizers On The Properties Of Edible Films Prepared From Fish Water-Soluble Proteins. Fisheries Science 67 (2001); 346 – 351.
17. Shi K., Huang Y., Yu H., Lee T., Huang Q. Reducing The Brittleness Of Zein Films Through Chemical Modification. Journal Of Agricultural And Food Chemisty 59 (2011); 56 – 61.
18. Rudnick J., Taylor P. Theory Of Free Volume In Polymers. Journal Of Polymer Science: Polymer Physics Edition 17 (1979); 311 – 320.
19. Sothornvit R., Krochta J. Plasticizer Effect On Mechanical Properties Of ß-Lactoglobulin Films. Journal Of Food Engineering 50 (2001); 149 – 155.
20. Dangaran K., Krochta J. Preventing The Loss Of Tensile, Barrier And Appearance Properties Caused By Plasticiser Crystallisation In Whey Protein Films. International Journal Of Food Science And Technology 42 (2007); 1094 – 1100.
21. Khodaverdi E., Mirzazadeh F., Amoli S., Sadeghi F. Comparison Of Plasticizer Effect On Thermo-Responsive Properties Of Eudragit RS Films. AAPS Pharmascitech 13(3) (2012); 1024 – 1030.
22. Huyng P., Takhistov P. Effect Of The Plasticizer On Phase Separation, Mechanical And Release Properties In API Laden Therapeutic Films. Poster Session Presented At The AAPS Meeting. San Antonio, TX. November (2013).
23. Donhowe I., Fennema O. The Effects Of Plasticizers On Crystallinity, Permeability, And Mechanical Properties Of Methylcellulose Films. Journal Of Food Processing And Preservation 17 (1993); 247 – 257.
24. Jangchud A., Chinnan M. Properties Of Peanut Protein Film: Sorption Isotherm And Plasticizer Effect. Food Science And Technology-Lebensmittel-Wissenschaft & Technologie 32 (1999); 89 – 94.
25. Chen H. Rheological Properties Of HPMC Enhanced Surimi Analyzed By Small- And Large-Strain Tests: I. The Effect Of Concentration And Temperature On HPMC Flow Properties. Food Hydrocolloids 21 (2007); 1201 – 1208.

26. Chen H., Huang Y. Rheological Properties Of HPMC Enhanced Surimi Analyzed By Small- And Large-Strain Tests—II: Effect Of Water Content And Ingredients. Food Hydrocolloids 22 (2008); 313 – 322.

27. Lin S., Amidon G., Weiner N., Goldberg A. Viscoelasticity Of Cellulose Polymers And Mucociliary Transport On Frog Palates. International Journal Of Pharmaceutics 95 (1993); 57 – 65.

28. Zangi R., Berne B. Aggregation And Dispersion Of Small Hydrophobic Particles In Aqueous Electrolyte Solutions. The Journal Of Physical Chemistry B 110 (2006); 22736 – 22741.

29. Robinson D., Grant M. The Effects Of Aqueous Salt Solutions On The Activity Coefficients Of Puke And Pyrimidine Bases And Their Relation To The Denaturation Of Deoxyribonucleic Acid By Salts. The Journal Of Biological Chemistry 241(17) (1966); 4030 – 4042.

30. Bertuzzi A., Gottifredi J. Mechanical Properties Of A High Amylose Content Corn Starch Based Film, Gelatinized At Low Temperature. Brazilian Journal Of Food Technology 15(3) (2012); 219 – 227.

31. Laboulfie F., Hemati M., Lamure A., Diguet S. Effect Of The Plasticizer On Permeability, Mechanical Resistance And Thermal Behaviour Of Composite Coating Films. Powder Technology 238 (2013); 14 – 19.

5. CONCLUDING REMARKS

Biopolymer films formulations have been extensively proposed as food coating and drug delivery methods. Many formulations have in common the use of a biocompatible polymer, able to form physical or chemical stable gels structures. In the pharmaceutical industry, the constant development of new drugs require in many cases, novel drug delivery methods able to offer long term stability of the pharmaceutical product and increase its bioavailability. The encapsulation and controlled releases of drugs in gel matrices is proposes as an innovative strategy able to fulfill these requirements.

Formulations of edible polymeric thin films have in common many formulation components. In this study the addition of flavorant agents, plasticizers and highly hydrophobic drug particles to non-ionic hydroxypropyl methylcellulose solutions was evaluated. The gelation temperature of the polymer-additive samples was the selected control variable, because is a key processing parameter directly related to the final product microstructure (morphology, fragility and permeability of the films). Rheological measurements are a verified method for the determination of this processing parameter, and were used in this work for the polymer solutions characterization.

The addition of flavorants, plasticizers and the active pharmaceutical ingredients to the polymer precursor solutions, triggered changes in the gelation temperature of the system

due to molecular interactions between the additives and the polymer molecules, and changes in the solubilizing properties of the solvent mixture.

Flavorants are small partially soluble molecules used to masking the bitter taste of some drugs. Due to solubility limitations, the flavorants concentrations evaluated in chapter 1 were considerably low (concentrations bellow 0.1%). Solubility and Flory interaction parameters were calculated for each flavorant concentration; however the results shown little variation between each other, and statistically it can be concluded that the flavorant concentration has no or little effect on these parameters. But, these slight changes in the concentration have a significant effect on the gelation temperature of HPMC solutions and the results can be used for the prediction of the behavior of slightly flavored film formulations, especially for pediatric use. When little or no change in the gelation temperature of HPMC solutions is required, the use of ester flavorants compounds are recommended as these molecules does not participate directly in the gelation mechanism of HPMC.

Small soluble molecules as menthyl acetate, ethyl cinnamate and terpinyl acetate are able to form H-H bonds with water, while the organic part of the molecule is capable of disrupting the water network continuity, promoting the hydrophobic interaction between the polymer chains, and therefore promoting gelation. It can be said that these compounds have a lubricant effect on the polymer chains, and contribute to the polymer chains

stretching. More compatibility and molecular interactions studies are required to evaluate the joint effect of flavorants in presence of more additives.

Hydrophobic interactions prevail when BCS class II drug particles were incorporated in HPMC solutions. Polymer absorption on the drug surface occurs because the polymer molecules have relatively long hydrophobic branches (~6Å), which could potentially provide significantly strong van der Wall forces with the drug hydrophobic surface. These polymer-polymer and polymer-particle interactions are highly dependent on the chain length and R_g (radius of gyration), since small polymer chains can be absorbed completely on the drug surface limiting the interactions with other polymer chains and reducing the probability of form entanglements.

Polymer films provide long term stability for pharmaceutical products; however one of its disadvantages is the maximum load that can be incorporated. Experimental results for griseofulvin and 1% HPMC showed that above 5% of drug concentration the gel matrix collapses, and the drug particles precipitate. These results are valuable for the development of a drug encapsulation system with similar properties, considerations about particle size and surface charge of the components are recommended. For suspend higher concentrations of drug particles a higher concentration of polymer may be used, however the effect of this modifications in the morphology and mechanical properties of the resulting films has to be evaluated.

The morphology and mechanical properties of the edible thin films based on a non-ionic biopolymer matrix as HPMC can be modified with the incorporation of plasticizers. These compounds are used as viscosity modifiers and may have a solubilizing or coagulant effect on the polymer solution. Glycerol as plasticizer, have a coagulant effect and promote the polymer-polymer interactions by reducing the solubility of the polymer in the aqueous medium. The integration of this plasticizer in a film formulation enhances the flexibility and reduces the brittleness of the product. Nonetheless, the addition of these additives may decrease the mechanical properties of the films when the used concentration promotes the aggregation of the plasticizer molecules; this generates plasticizer rich zones with different mechanical resistance to stress than the rest of the film. Rheological measurements were successfully implemented as a screening tool for phase separation determination in HPMC-glycerol films, this method proved to be a fast and efficient way to build and control the quality of the films in real time industrial applications.

6. APPENDIX

6.1 Appendix from the chapter 2

Linear regression analyses were performed for all the HPMC-flavorant solutions. The effect of the type of flavorants (by groups) and the effect of the concentration were evaluated.

For all functional groups equal variance tests (parametric and non-parametric) were performed to evaluate the data consistency.

6.1.1 Alcohol Flavorants

Equal variance tests were performed to evaluate the data consistency. Bartlett's and Levene's test are used to evaluate the variance distribution for both parametric and non-parametric. P-values above 0.05 indicate a consistent equal variance for all samples.

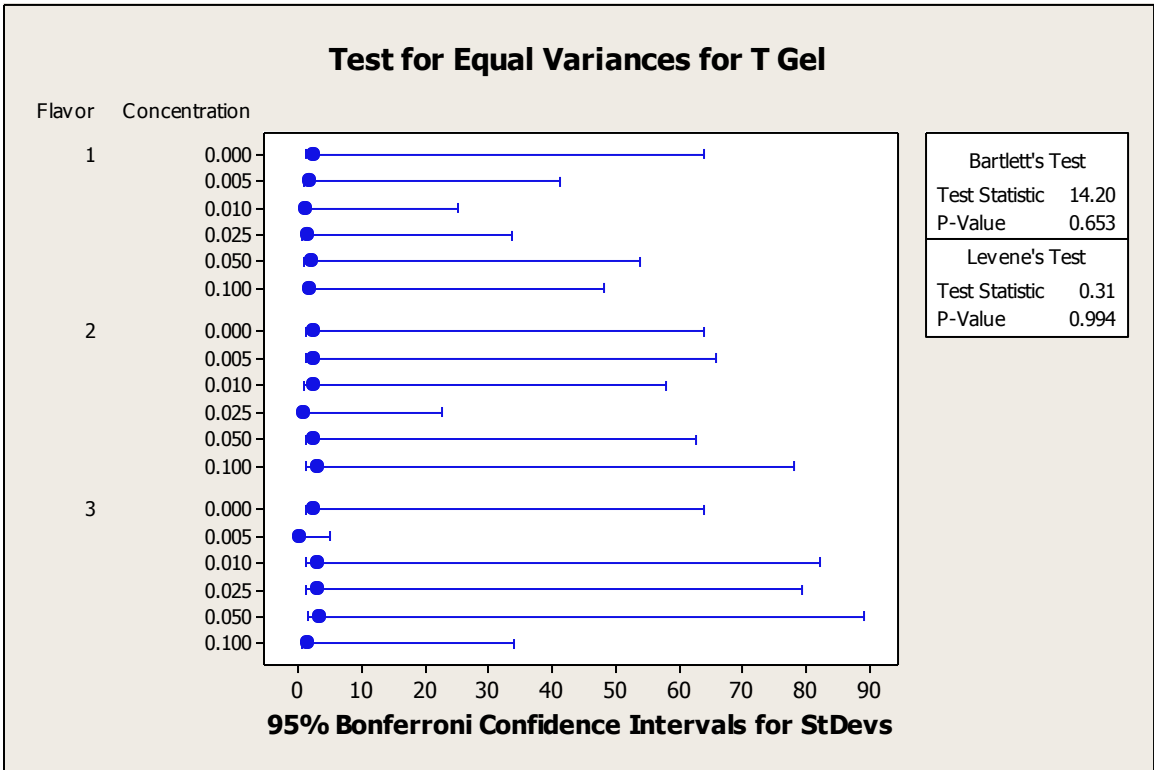


Figure 53. Test for equal variance for alcohol flavorants (1. cinnamyl alcohol. 2. eugenol. 3. citronellol)

Two-way ANOVA: T Gel versus Flavor, Concentration

Source	DF	SS	MS	F	P
Flavor	2	203.717	101.859	21.52	0.000
Concentration	5	378.300	75.660	15.98	0.000
Interaction	10	67.763	6.776	1.43	0.206
Error	36	170.417	4.734		
Total	53	820.197			

S = 2.176 R-Sq = 79.22% R-Sq(adj) = 69.41%

Results from the ANOVA analysis confirm that the type of flavorant and concentration, both have a significant effect on the gelation temperature of HPMC solutions. However, the interaction of factors does not has a significant effect.

Figure 54 was generated to illustrate the interaction of factors and its effect on T_{gel}. In the 3D plot no significant peaks or valleys are observed (sudden inflexions) so it can be concluded (jointly with the p-value obtained in the ANOVA) that the interaction of factors has no significant effect on T_{gel}.

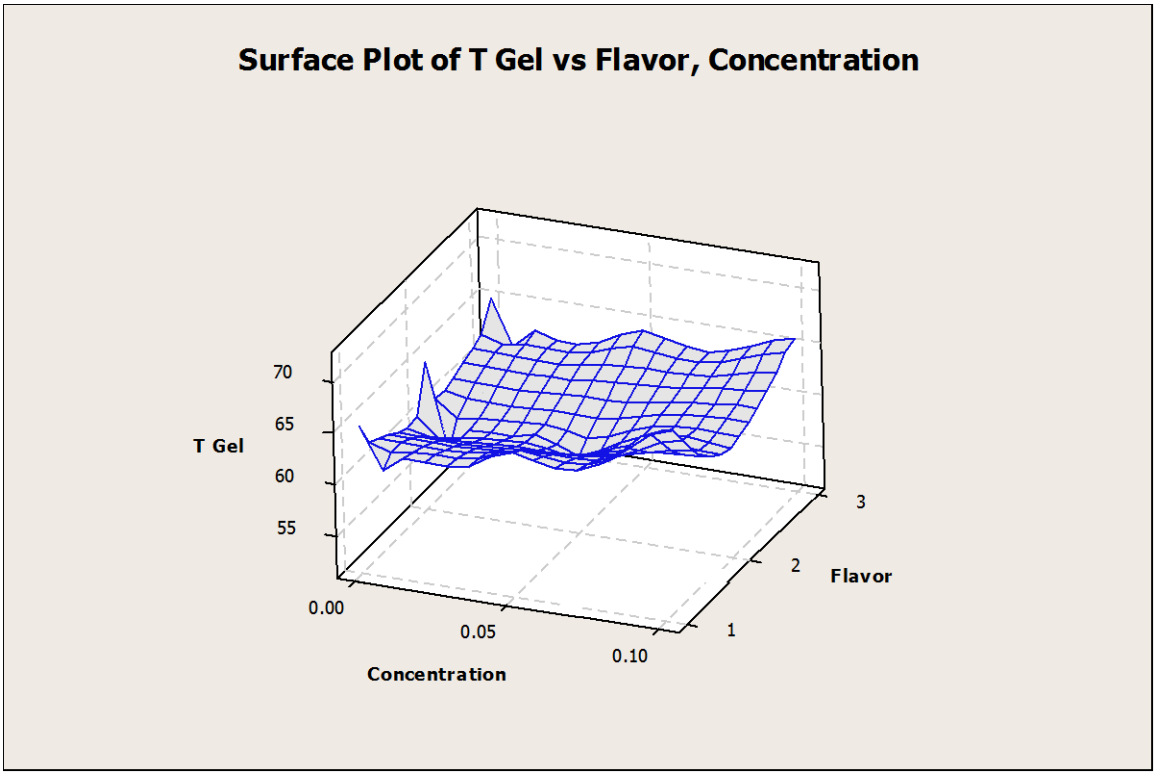


Figure 54. Surface plot. Effect of the interaction of factors on Tgel (flavor – concentration) for alcohol flavorants

6.1.2 Ester flavorants

Same analyses were performed for all functional groups. Equal variance tests, ANOVA analysis and interaction of factors analysis were performed. A graphical summary is shown below.

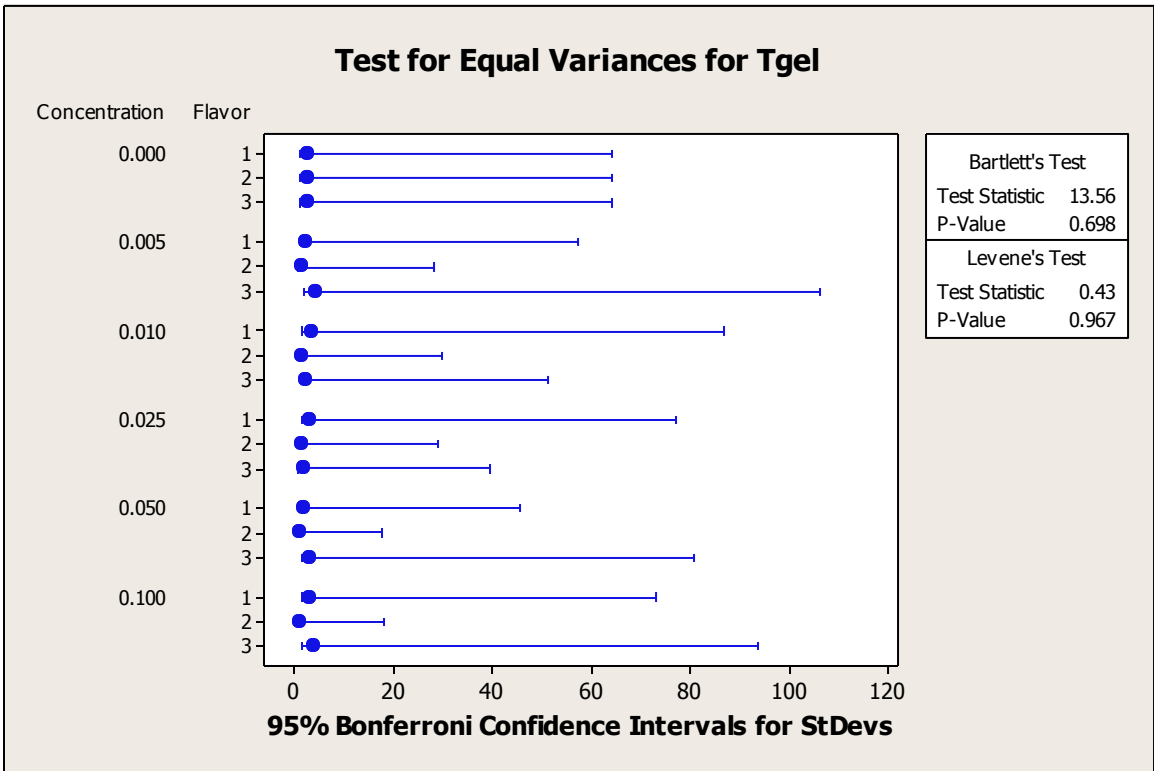


Figure 55. Test for equal variance for ester flavorants (1. ethyl cinnamate. 2. menthyl acetate. 3. terpinyl acetate)

Two-way ANOVA: Tgel versus Flavor, Concentration

Source	DF	SS	MS	F	P
Flavor	2	1.670	0.8351	0.15	0.858
Concentration	5	60.901	12.1801	2.25	0.070
Interaction	10	59.933	5.9933	1.11	0.384
Error	36	195.047	5.4180		
Total	53	317.550			

S = 2.328 R-Sq = 68.58% R-Sq(adj) = 69.57%

From the ANOVA results it can be concluded that no significant effect for flavorant, concentration or the interaction is present for ester flavorants.

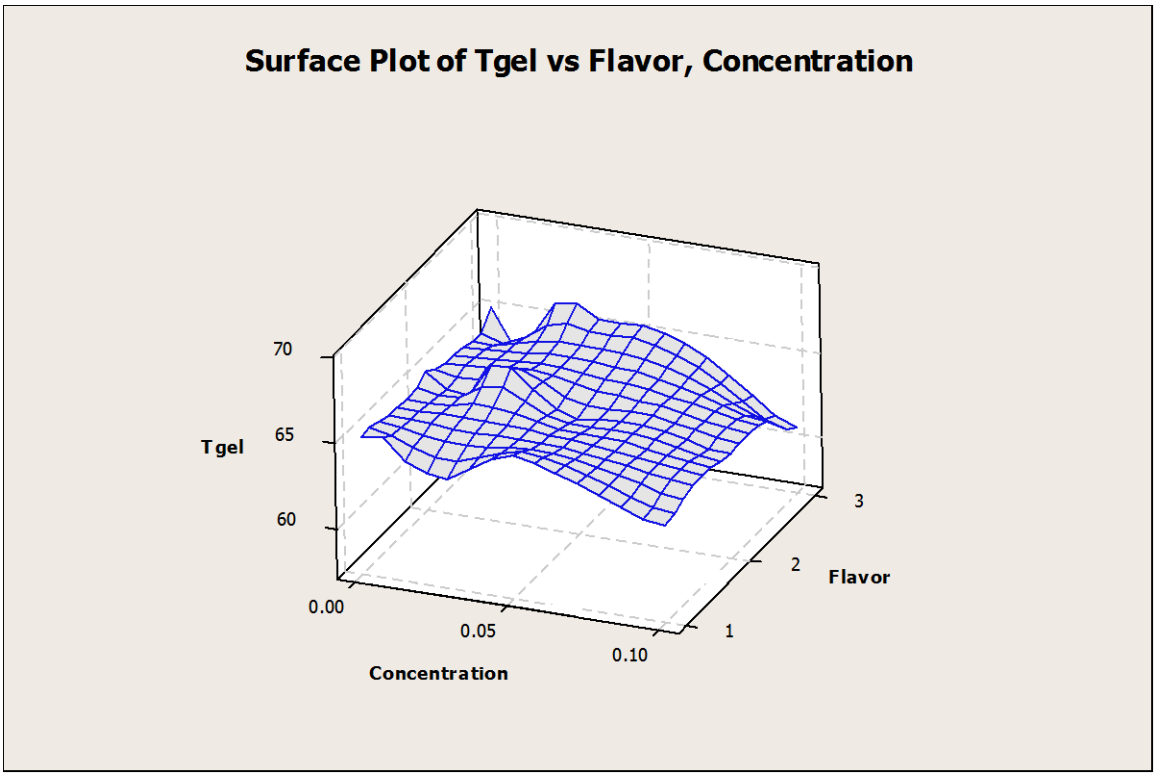


Figure 56. Surface plot. Effect of the interaction of factors on Tgel (flavor – concentration) for ester flavorants

6.1.3 Aldehyde flavorants

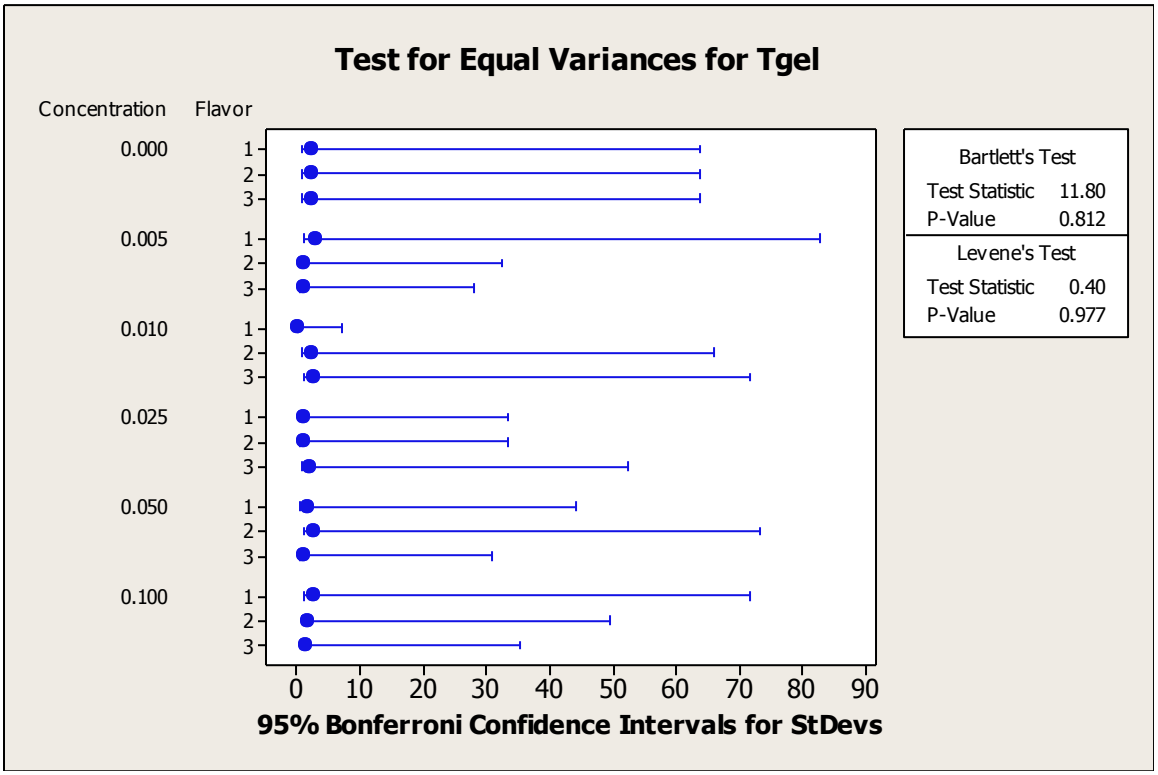


Figure 57. Test for equal variance for aldehyde flavorants (1. vanillin. 2.cinnamaldehyde. 3. ethyl vanillin)

P-values above 0.05 for the equal variance test indicate consistent variance for all samples.

Two-way ANOVA: Tgel versus Flavor, Concentration

Source	DF	SS	MS	F	P
Flavor	2	176.916	88.4579	21.79	0.000
Concentration	5	163.667	32.7334	8.06	0.000
Interaction	10	137.131	13.7131	3.38	0.003
Error	36	146.119	4.0589		
Total	53	623.833			

S = 2.015 R-Sq = 76.58% R-Sq(adj) = 65.52%

Significant effect is observed for both flavorant and concentration, as well as for the interaction.

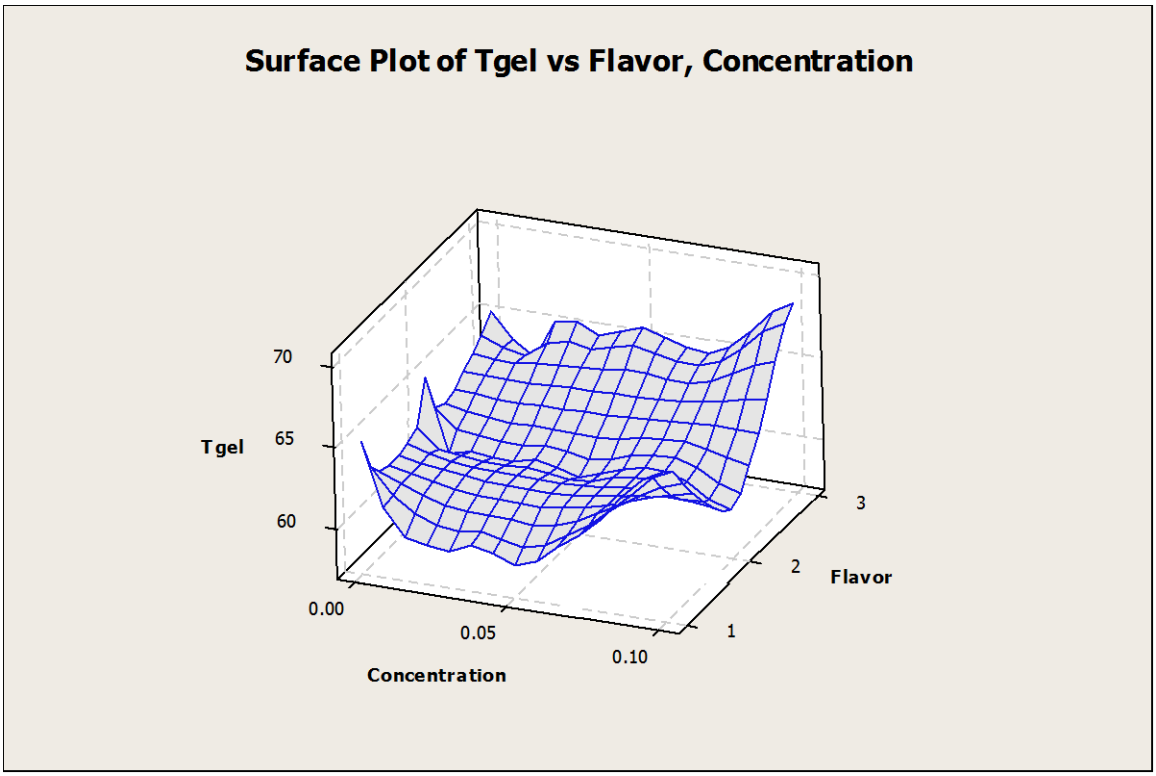


Figure 58 Surface plot. Effect of the interaction of factors on Tgel (flavor – concentration) for ester flavorants

6.1.4 Monoterpene flavorants

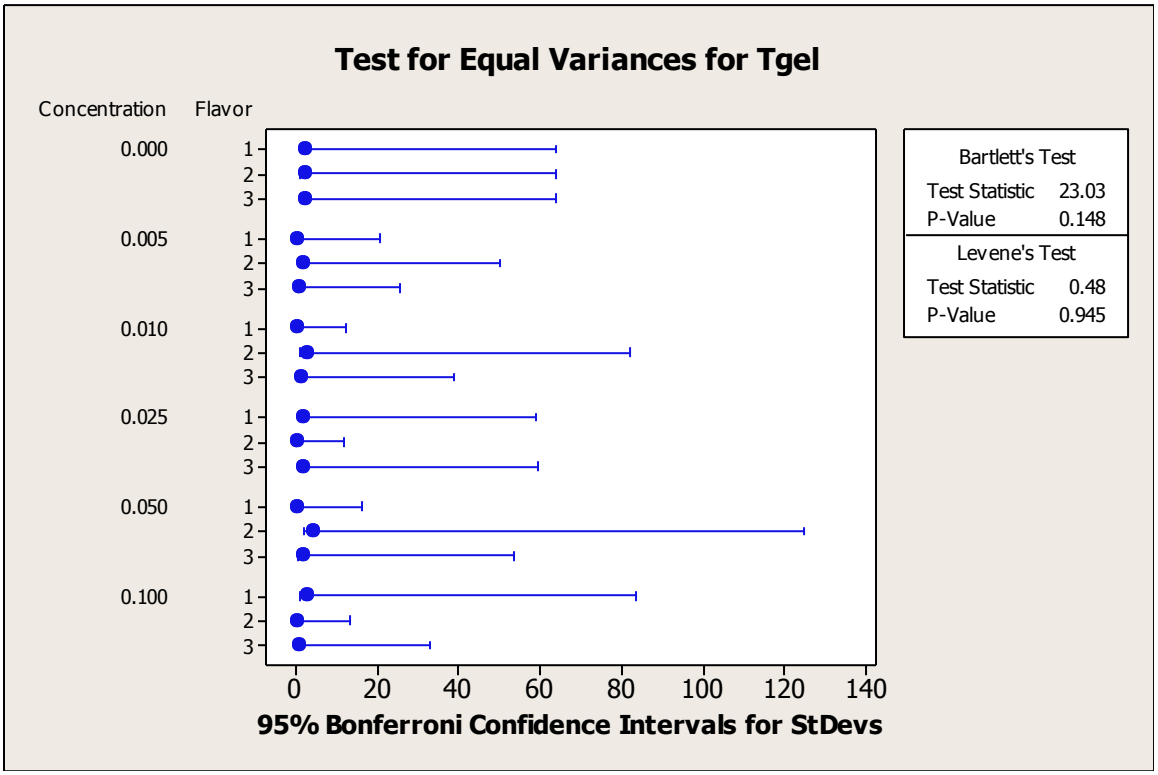


Figure 59. Test for equal variance for monoterpene flavorants (1. menthol. 2.geraniol. 3. eucalyptol)

From the equal variance test p-values above 0.05 indicate a variance constant for all samples.

Two-way ANOVA: Tgel versus Flavor, Concentration

Source	DF	SS	MS	F	P
Flavor	2	299.830	149.915	33.25	0.000
Concentration	5	55.619	11.124	2.47	0.051
Interaction	10	161.516	16.152	3.58	0.002
Error	36	162.313	4.509		
Total	53	679.278			

S = 2.123 R-Sq = 76.11% R-Sq(adj) = 64.82%

According to the ANOVA results, the flavorant, the concentration and the interaction have significant effect on T_{gel} for monoterpene flavorant.

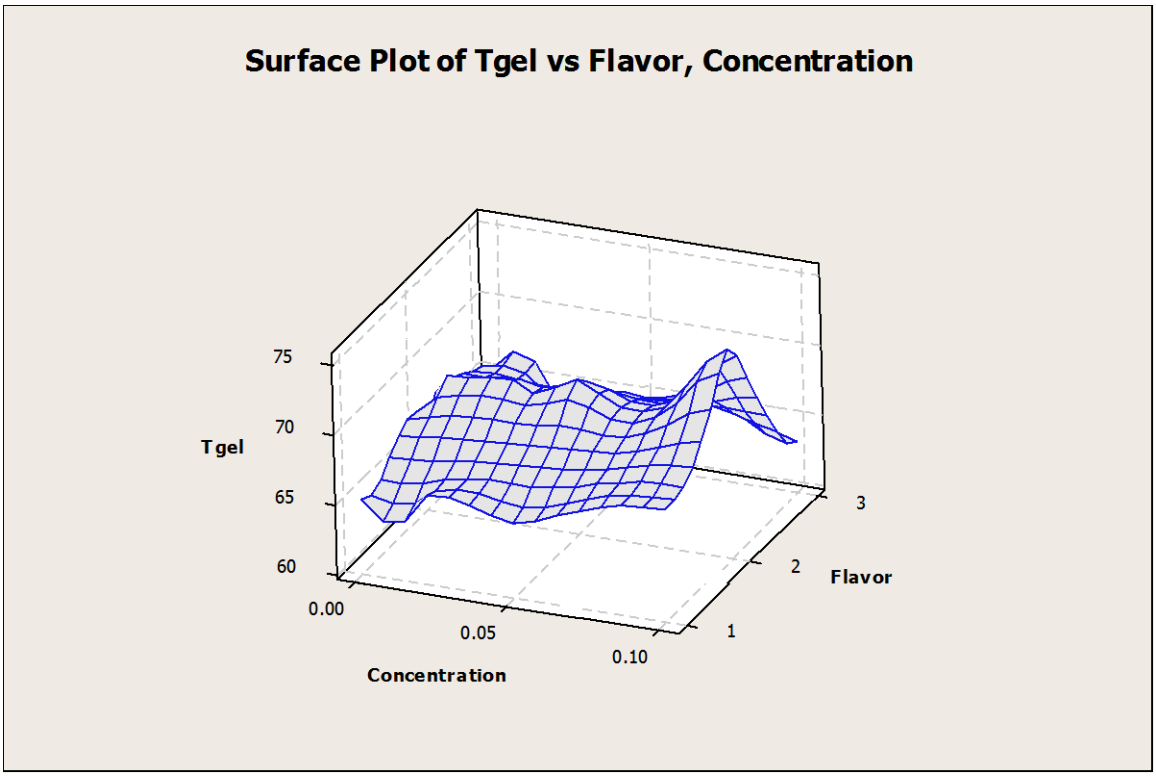


Figure 60. Surface plot. Effect of the interaction of factors on Tgel (flavor – concentration) for ester flavorants

For geraniol and menthol (alcohol-like monoterpenes) a second ANOVA was performed to evaluate the concentration effect on T_{gel}. From the Minitab results it can be concluded that both flavorants have a significant effect on Tgel for HPMC solutions in the studied concentration range.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Flavor	1	212.67	212.674	34.97	0.000
Conc	5	127.75	25.550	4.20	0.005
Error	29	176.35	6.081		
Lack-of-Fit	5	52.21	10.442	2.02	0.112
Pure Error	24	124.14	5.172		
Total	35	516.77			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.46596	65.87%	58.81%	47.41%

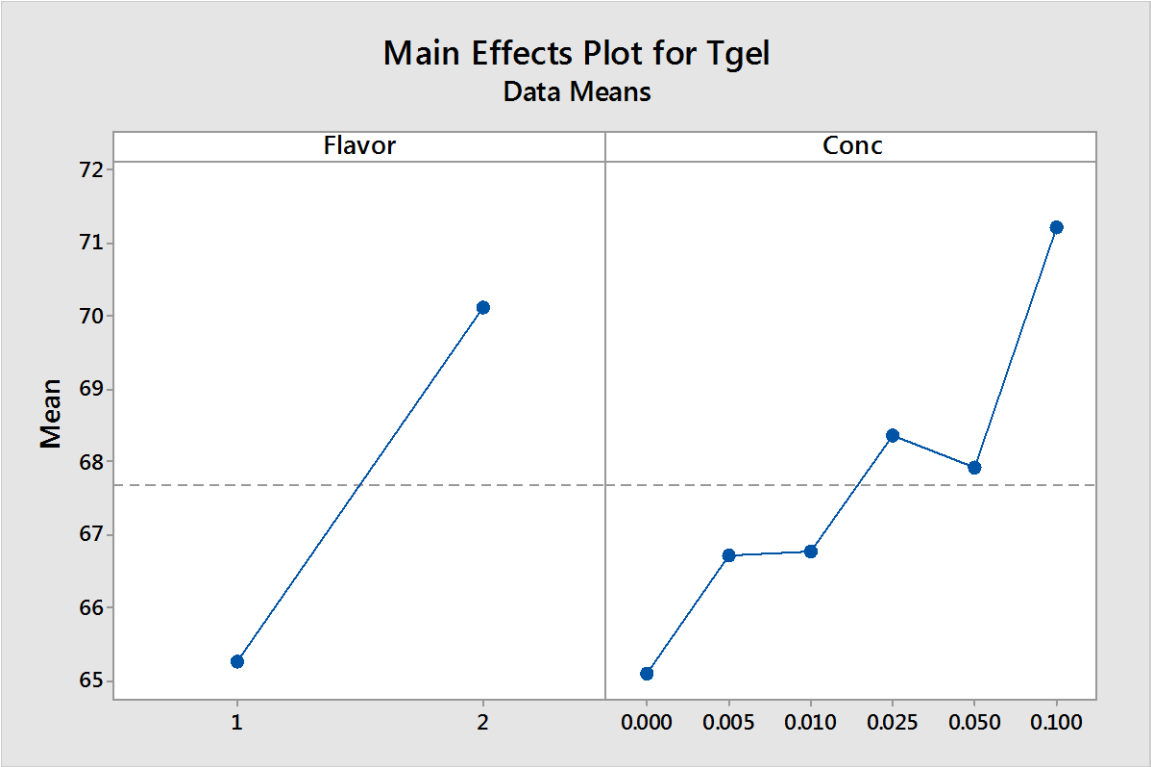


Figure 61. Main effects plots for evaluated factors (flavor and concentration) in menthol and geraniol – HPMC solutions

The main effects plot show a significant effect for both factors evaluated.

6.2 Appendix from the chapter 3

The data distribution for GF and FNB is shown on Figure 62. From the graphical summary on the right side of Figure 55 and the Anderson-Darling normality test results it can be deduced that the T_{gel} data does not have a normal distribution, especially the data for FNB which have a p-value below 0.005. For FNB a few outliers can be observed, but these values represents the T_{gel} values of HPMC solutions without drug. Outliers are odd

values in the data; however these values being the 0% concentration does not affect the data consistency.

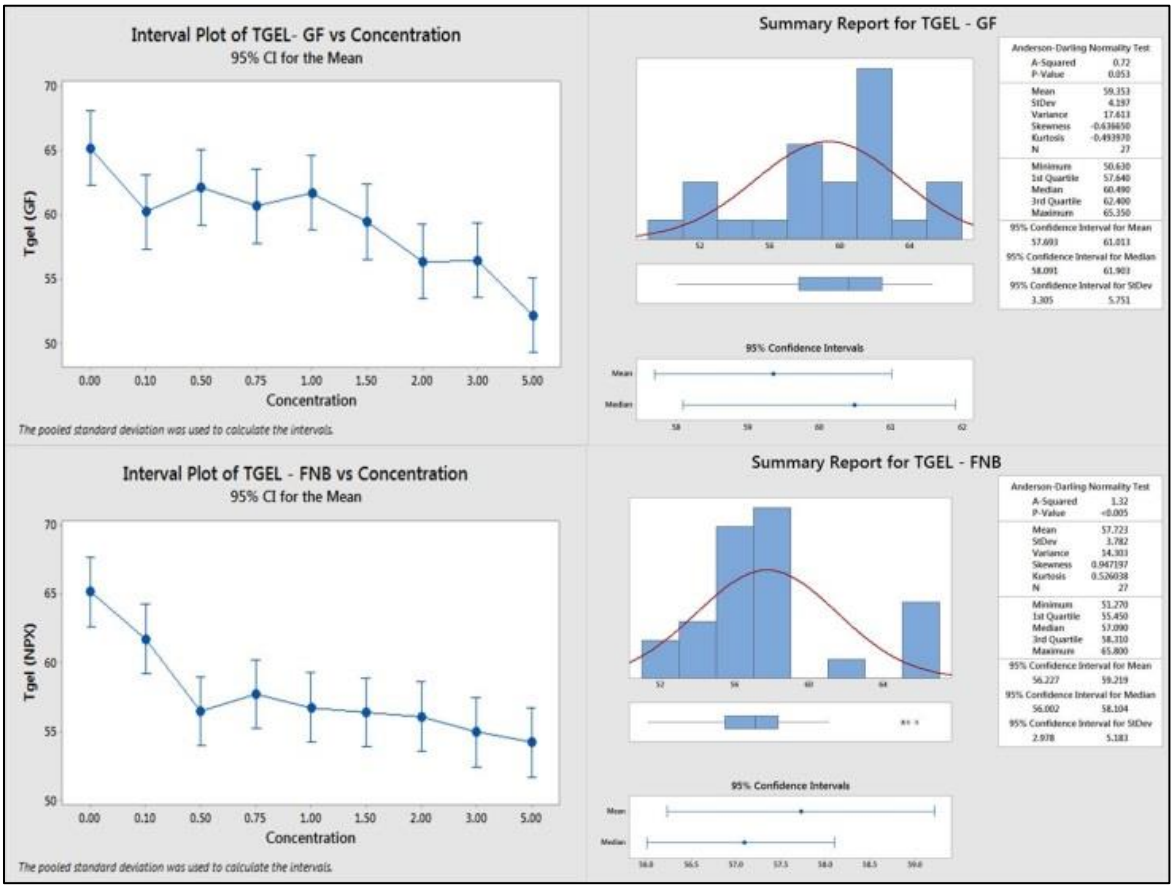


Figure 62 .Graphical summary of T_{gel} data for GF and FNB in HPMC E4M solutions

A global ANOVA for all the data (GF and FNB) allow us to conclude that the drug concentration has a significant effect in T_{gel}, with p-values below 0.001as shown on Figure 63. The Anderson-Darling test has a p-value of 0.318 which means that the data have a normal distribution with a slight negative kurtosis; this means that the data is slightly

distributed more to the right of the histogram which indicates that most of the data for Tgel is in the range of 58 to 62 °C and the effect of the addition of the drugs is mostly significant at low concentrations.

It can be concluded from this section that the concentration of drug particles, have a significant effect on the gelation process of HPMC solutions. Despite HPMC solutions with presence of BCS Class II drugs having a higher mechanical stability due to drug-polymer interactions that promote the gelation, above 5% (for GF) exhibited gel collapse.

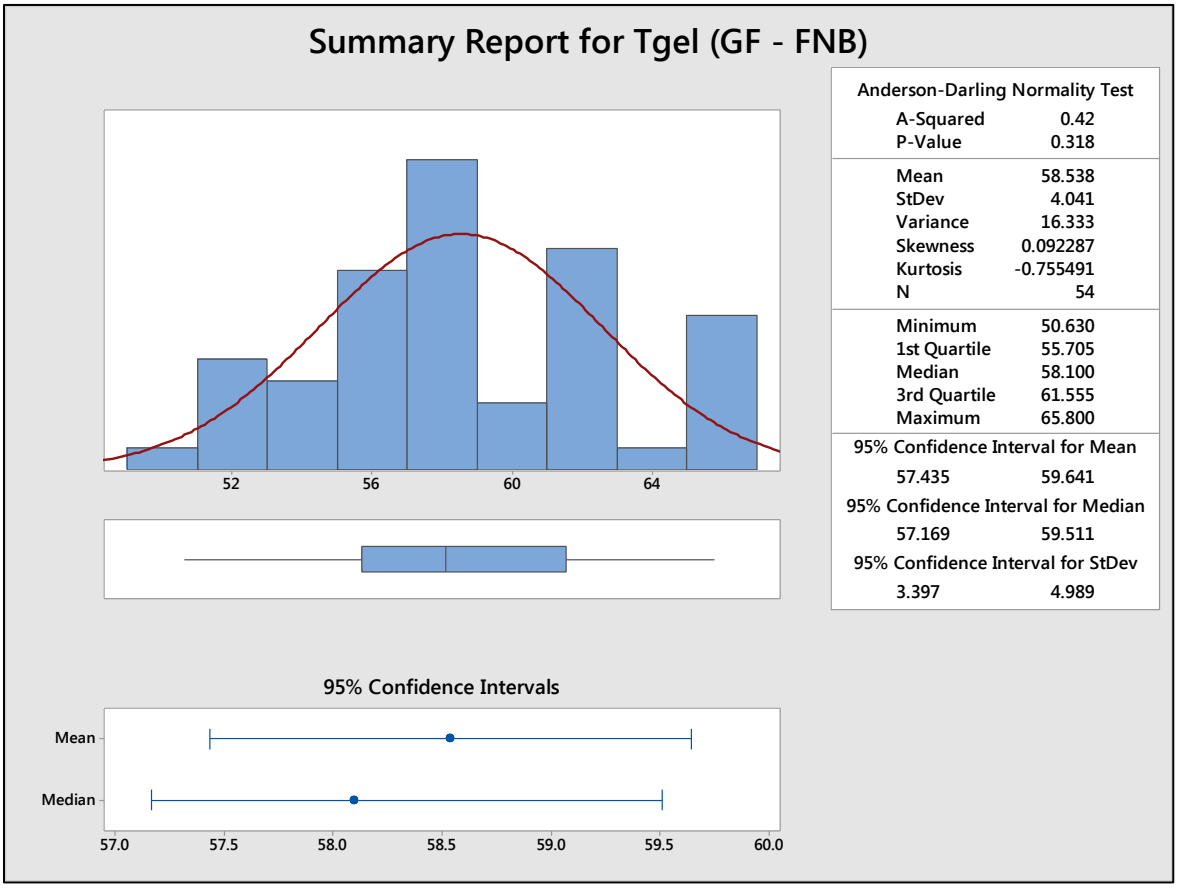


Figure 63. Graphical summary of T_{gel} data for all concentrations for both drugs GF – FNB.

Two different ANOVAs were performed to evaluate the effect of the addition of BCS Class II drugs to HPMC solutions. First all data was evaluated from 0 to 5 (drug/polymer ratio). Then, a second ANOVA (for GF and FNB) was performed eliminating the values below 0.5 (where the bigger effect is observed).

From the First ANOVA for FNB a significant effect of the concentration of the drug is observed on T_{gel}. A p-value significantly lower than 0.05 was obtained.

```
Analysis of Variance (FNB all concentrations)
*
Source  DF  Adj SS  Adj MS  F-Value  P-Value
C1       8  294.31  36.788     8.54    0.000
Error   18   77.56   4.309
Total   26  371.87


Model Summary
      S    R-sq  R-sq(adj)  R-sq(pred)
2.07585  79.14%     69.87%      53.07%
```

Removing the data for 0, 0.1, and 0.5% FNB, the results still indicate a no relation between T_{gel} and the drug concentration. This is because the higher effect on Tgel is noted at low drug concentrations (for FNB).

```
Analysis of Variance (FNB without concentrations below 0.05)

Source  DF  Adj SS  Adj MS  F-Value  P-Value
C3       6   24.41   4.068     1.17    0.377
Error   14   48.81   3.487
Total   20   73.22


Model Summary
      S    R-sq  R-sq(adj)  R-sq(pred)
1.86722  33.33%     74.76%      70.00%
```

In the case of GF, a significant effect on Tgel by changes in the concentration of the drug is observed in both ANOVAs, with and without the concentrations below 0.05%. This

indicates that HPMC have a more strong affinity with GF particles and the effect of the addition of particles is constant in the studied concentration range.

Analysis of Variance (GF all concentrations)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
cONC	8	355.4	44.419	7.79	0.000
Error	18	102.6	5.699		
Total	26	457.9			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.38724	77.60%	67.64%	49.60%

Analysis of Variance (GB without concentrations below 0.05)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
CONCE	6	232.94	38.824	6.26	0.002
Error	14	86.82	6.201		
Total	20	319.76			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.49020	72.85%	61.21%	58.91%

For the evaluation of the effect of the molecular weight on T_{gel} two separate ANOVAs were performed (one for each drug). For GF a significant effect of the molecular weight on T_{gel} was observed, with a p-value below 0.05.

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Polymer	1	931.09	931.094	103.36	0.000
Concentration	8	442.32	55.289	6.14	0.000
Error	44	396.37	9.008		
Lack-of-Fit	8	99.66	12.458	1.51	0.188
Pure Error	36	296.71	8.242		
Total	53	1769.78			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
3.00140	77.60%	73.02%	66.27%

In Figure 64 it can be observed, a trend to increase T_{gel} from E4M to E15LV for all GF concentrations.

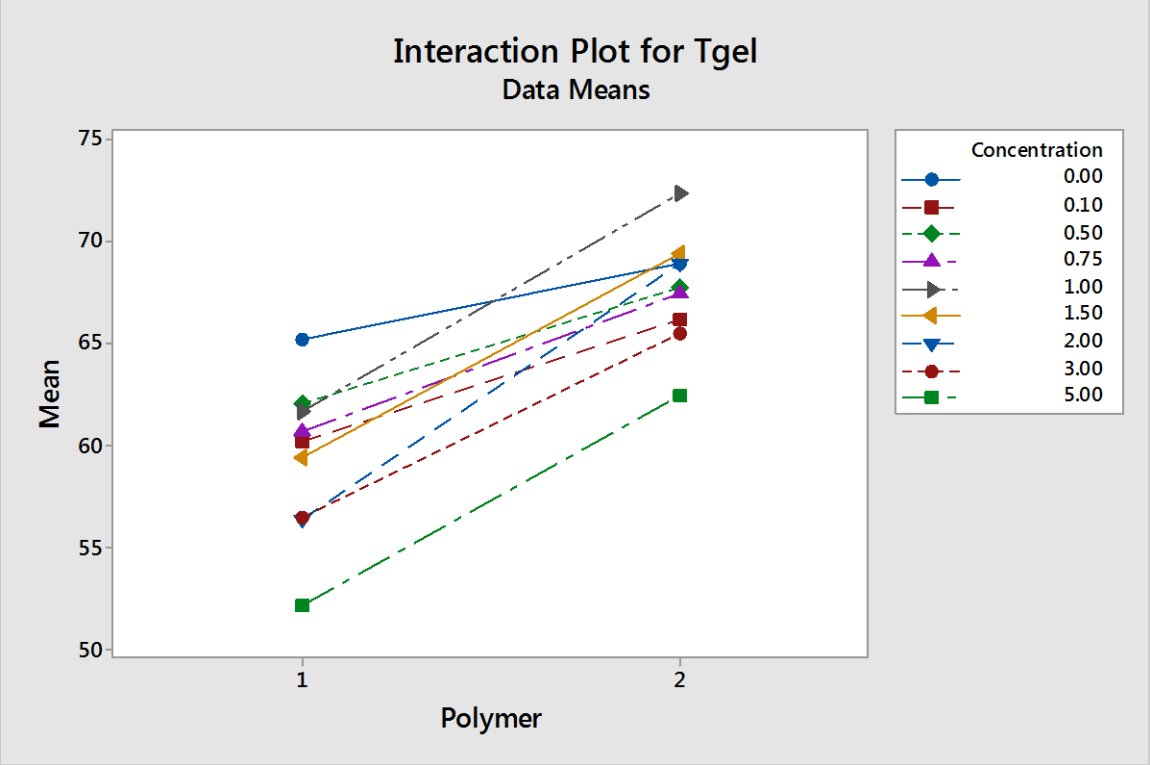


Figure 64. Interaction plot for GF-HPMC for both molecular weight (1. E4M, 2. E15LV)

This plot shows a clear trend to increase T_{gel} when decreasing the molecular weight of the polymer for HPMC-GF solutions.

For FNB the effect of the molecular weight is almost no significant with a p-value of 0.052. However a trend to increase T_{gel} when decreasing the molecular weight is present in almost all samples. The main effect is observed in concentrations below 2%. (Figure 65).

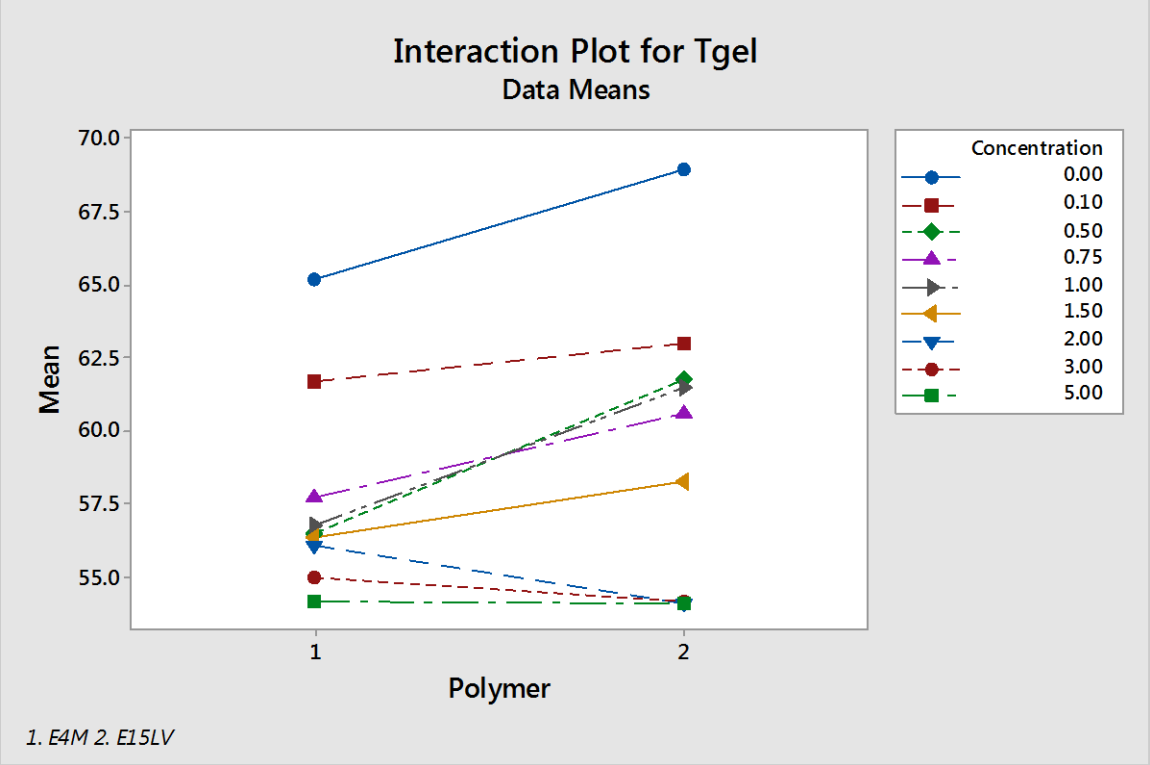


Figure 65. Interaction plot for GF-HPMC for both molecular weight (1. E4M, 2. E15LV)

```
Analysis of Variance

Source           DF   Adj SS   Adj MS  F-Value  P-Value
  Polymer         1    48.05   48.053     4.00    0.052
  Concentration   8   815.82  101.977     8.48    0.000
Error            44   528.96   12.022
  Lack-of-Fit     8    75.76    9.470     0.75    0.646
  Pure Error     36   453.20   12.589
Total            53  1392.83


Model Summary
      S    R-sq  R-sq(adj)  R-sq(pred)
3.46724  62.02%     54.25%      42.80%
```

6.3 Appendix from the chapter 4

The effect of the type of plasticizer and its concentration was evaluated by linear regression modeling. Results are summarized in the ANOVA below.

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	3	2574.0	857.98	65.43	0.000
Concentration	1	1574.3	1574.33	120.07	0.000
Plasticizer	2	999.6	499.81	38.12	0.000
Error	68	891.6	13.11		
Lack-of-Fit	20	564.2	28.21	4.14	0.000
Pure Error	48	327.4	6.82		
Total	71	3465.6			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
3.62110	74.27%	73.14%	71.01%

p-values below 0.05 for the concentration and type of plasticizer were obtained, indicating that both factors have a significant effect on the gelation temperature of HPMC solutions.

Data Validation – HPMC 5%. (Glycerol Concentration vs Viscosity & Yield Stress)

Table 11. Plateau viscosity/Yield stresses for all HPMC 5% - glycerol solutions

Glycerol Concentration (weight %)	Viscosity (Pa s)	Yield Stress (Pa)
0.00	0.117	1.009
0.00	0.117	1.008
0.00	0.117	1.001
0.55	0.122	1.005
0.55	0.121	1.008
0.55	0.121	1.006
1.00	0.122	1.004
1.00	0.121	1.005
1.00	0.121	1.003
2.00	0.126	1.002
2.00	0.125	1.005
2.00	0.126	1.009
3.00	0.135	1.009
3.00	0.135	1.005
3.00	0.135	1.011
5.00	0.142	1.005
5.00	0.144	1.003
5.00	0.142	1.002
8.00	0.144	1.003
8.00	0.144	1.006
8.00	0.145	1.002
10.00	0.173	1.008
10.00	0.173	1.003
10.00	0.173	1.012

ANOVA results for HPMC 5% - Glycerol solutions (Plateau Viscosity)

Source	DF	SS	MS	F	P
Glycerol Concentration	7	0.0099359	0.0014194	3457.92	0.000
Error	18	0.0000074	0.0000004		
Total	25	0.0099433			

S = 0.0006407 R-Sq = 99.93% R-Sq(adj) = 99.90%

ANOVA results for HPMC 5% - Glycerol solutions (Yield Stress)

Source	DF	SS	MS	F	P
Glycerol Concentration	7	0.0001158	0.0000165	2.04	0.036
Error	18	0.0001461	0.0000081		
Total	25	0.0002619			

S = 0.002849 R-Sq = 74.22% R-Sq(adj) = 72.52%

From the ANOVA analysis for HPMC 5% solutions, with the evaluated glycerol concentrations it can be concluded that the glycerol concentration have a significant effect on the viscosity and yield stress of the samples. From the Figures 66 and 67 it can be observed a normal data distribution for all viscosity and yield stress data respectively.

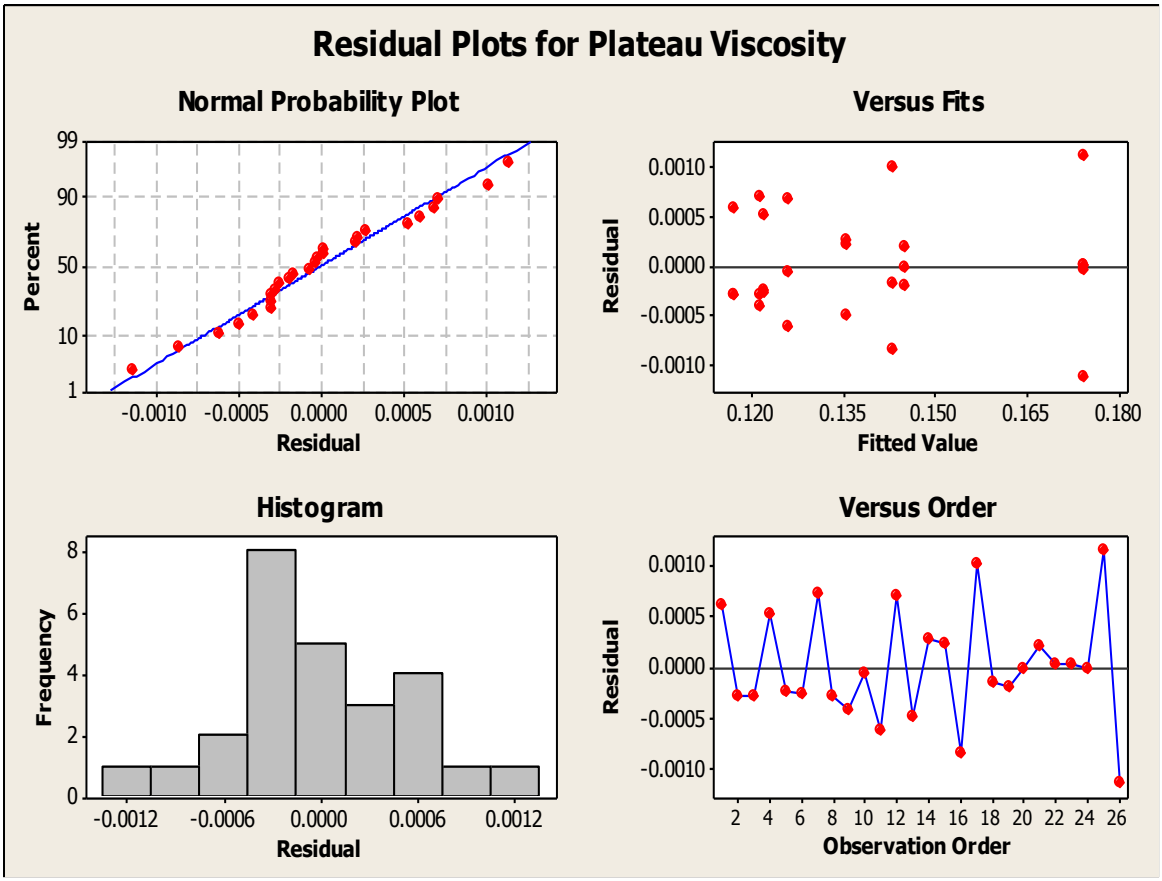


Figure 66. Residual Plots for Plateau viscosity HPMC 5% – All measurements

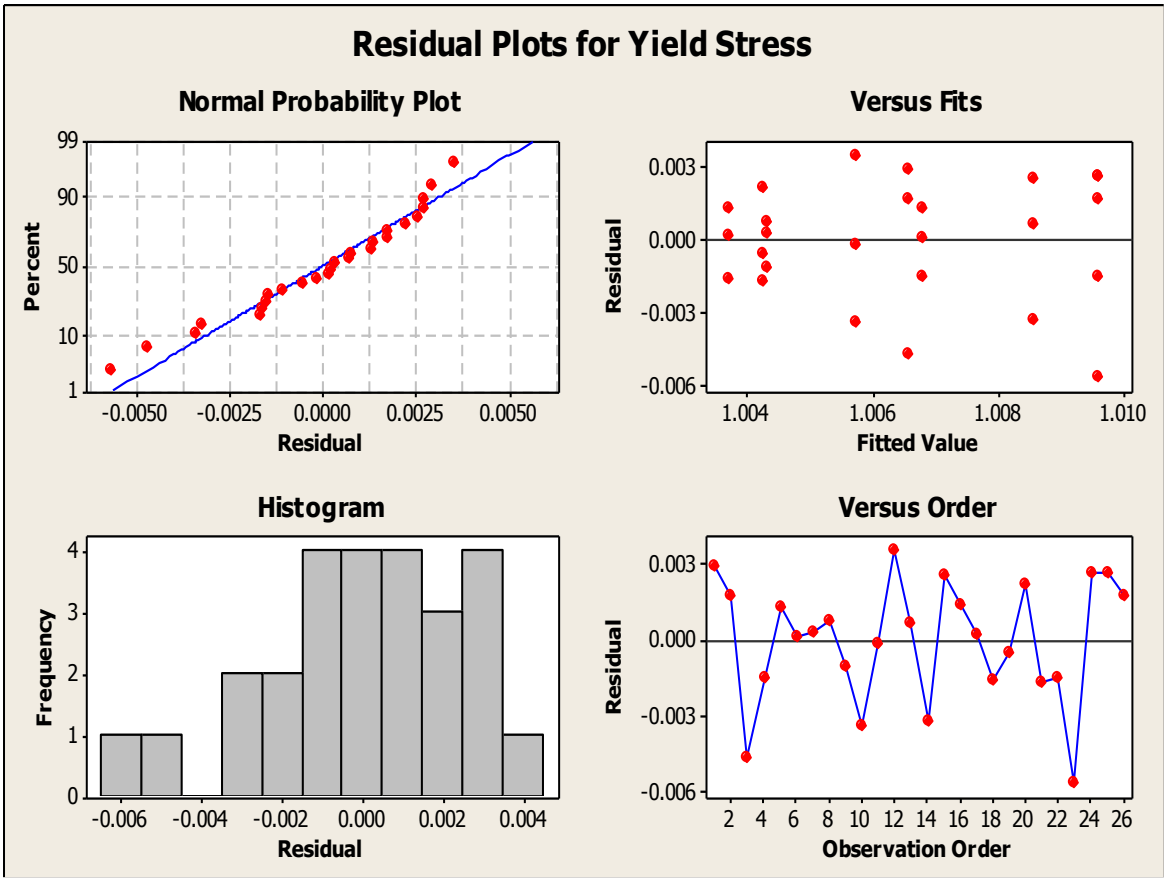


Figure 67. Residual Plots for Yield Stress HPMC 5% – All measurements

Data Validation – HPMC 2.5%. (Glycerol Concentration vs Viscosity & Yield Stress)

Table 12. Plateau viscosity/Yield stresses for all HPMC 2.5% - glycerol solutions

Glycerol Concentration (weight %)	Viscosity (Pa s)	Yield Stress (Pa)
0.00	0.018	1.003
0.00	0.016	1.000
0.00	0.016	1.001
0.55	0.019	1.003
0.55	0.017	1.001
0.55	0.017	1.000
1.00	0.017	1.004
1.00	0.016	1.000
1.00	0.016	1.001
2.00	0.018	1.000
2.00	0.018	1.000
2.00	0.018	1.005
3.00	0.021	1.005
3.00	0.020	1.000
3.00	0.020	1.000
5.00	0.021	1.001
5.00	0.021	1.001
5.00	0.021	1.000
8.00	0.023	1.005
8.00	0.022	1.001
8.00	0.022	1.005
10.00	0.025	1.000
10.00	0.026	1.003
10.00	0.024	1.003

ANOVA results for HPMC 2.5% - Glycerol solutions (Plateau Viscosity)

Source	DF	SS	MS	F	P
Glycerol Concentration	7	0.0001786	0.0000255	48.42	0.000
Error	16	0.0000084	0.0000005		
Total	23	0.0001870			

S = 0.0007259 R-Sq = 95.49% R-Sq(adj) = 93.52%

ANOVA results for HPMC 2.5% - Glycerol solutions (Yield Stress)

Source	DF	SS	MS	F	P
Glycerol Concentration	7	0.0000136	0.0000019	0.42	0.487
Error	16	0.0000743	0.0000046		
Total	23	0.0000879			

S = 0.002154 R-Sq = 75.47% R-Sq(adj) = 73.10%

From the ANOVA analysis for HPMC 2.5% solutions, with the evaluated glycerol concentrations it can be concluded that the glycerol concentration have a significant effect on the viscosity and yield stress of the samples with p-values of 0.000 and 0.487 respectively. From the Figures 68 and 69 it can be observed a normal data distribution for all viscosity and yield stress data respectively.

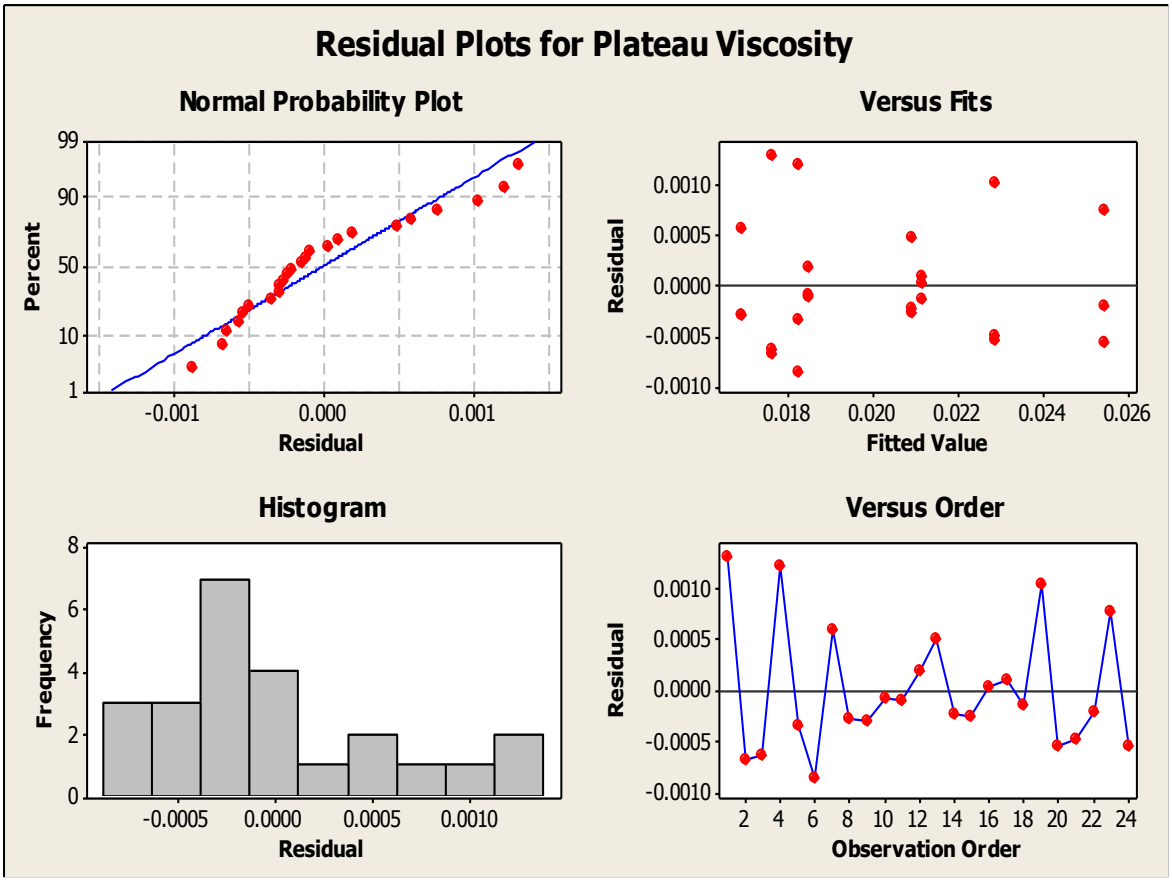


Figure 68. Residual Plots for Plateau viscosity HPMC 2.5% – All measurements

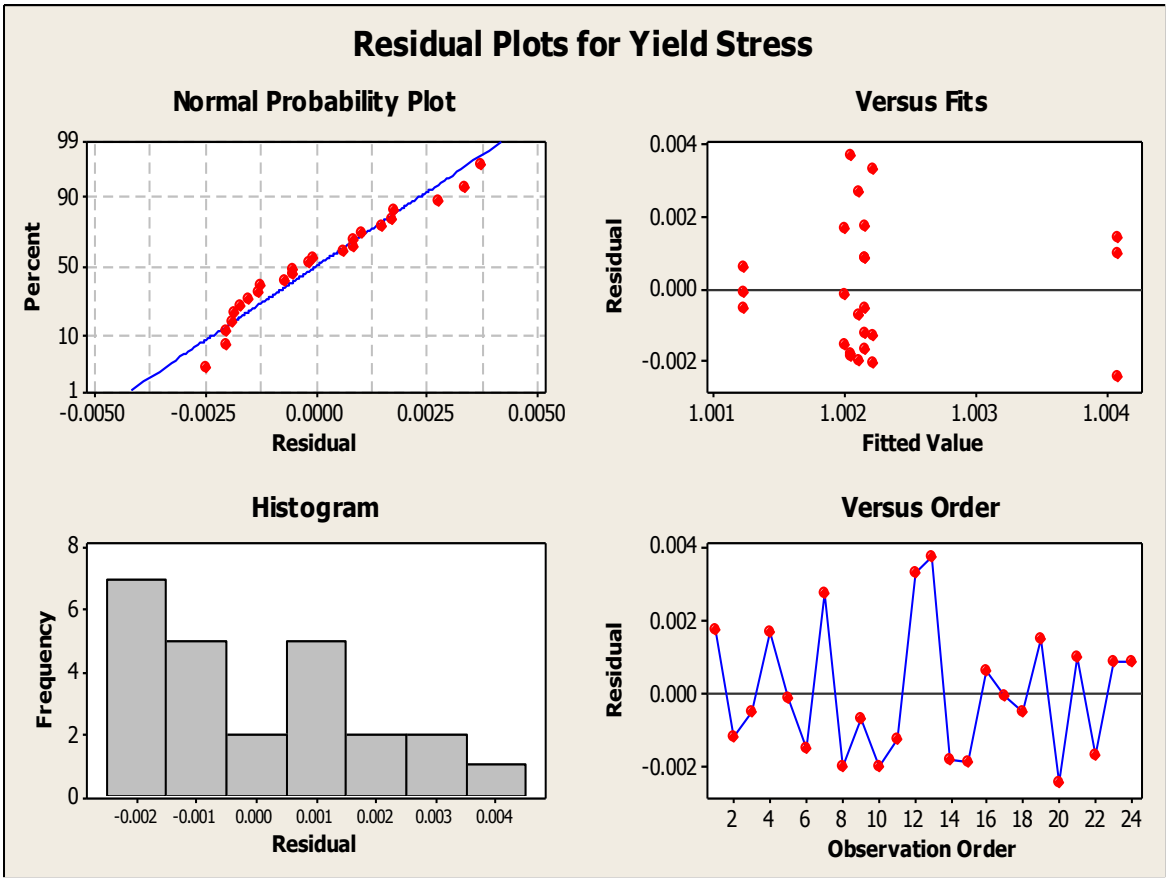


Figure 69. Residual Plots for Yield Stress HPMC 2.5% – All measurements

From the results of the statistical analysis for both 2.5 and 5% HPMC solutions it can be concluded that the glycerol concentration have a significant effect on the plateau viscosity and yield stress of the samples. Properties that were related in this chapter to changes in the morphological properties of HPMC/glycerol dry casted films.