# Viscoelastic properties of phosphoric and oxalic acid-based chitosan hydrogels.

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## Abstract

In a previous work [1], we have shown that chitosan physical gelation occurs with some organic and inorganic acids. Two acid-based systems presenting similar gelation mechanisms were chosen in order to investigate furthermore the sol-gel transition: the chitosan-phosphoric acid system, which can be considered for food applications, and the chitosan-oxalic acid system. We have studied the effect of parameters such as polymer concentration, ionic strength and counter-ion type on the evolutive properties of the gels by performing rheological measurements in the linear viscoelastic regime [2]. For both systems, gelation occurred for polymer concentrations above a critical concentration $C_{gel}$, around 5% w/v (g/100mL). Isothermal time sweep experiments showed that the gelation occurs in three stages: 1) incubation; 2) rapid increase of $G'$; and 3) a slow stage where $G'$ reached equilibrium due to slow molecular diffusion. Cooling-heating cycles under small amplitude oscillatory shear within the range 80-10°C revealed that the gels showed thermoreversibility after one cycle, but became permanent after the second cycle for chitosan-oxalic acid and the third for chitosan-phosphoric acid.

## Introduction

Acid solvent counter-ions can play a role in electrostatic charges screening and in some cases lead to physical gelation through a delicate interplay of molecular interactions. A study [1] has been conducted in our laboratories to investigate the feasibility of chitosan gelation by using food and non-food graded acids in aqueous solutions based on the pioneering work of Hayes and Davies [3] and Yamaguchi et al. [4] using oxalic acid. We have investigated a variety of monovalent and multivalent acids and compared the chain length, the acid type (organic and mineral), the counter-ion type ($Cl^-$, $COO^-$, $H_2PO_4^-$, $HSO_4^-$, $SO_4^{2-}$) and the chemical structure of the solvent on the possibility of achieving chitosan gelation. The study revealed that physical gelation was possible with three inorganic acids (sulfuric, phosphoric and hydrochloric) and one organic (oxalic) acid, confirming in the latter case the previous research ([3], [4]). Gelation was favored by simple and short chain length acids and was governed by ionic interactions.

In the present work, we report an investigation of the sol-gel transition and viscoelastic properties of the two hydrogels based on weak multivalent acids: the chitosan-phosphoric acid and the chitosan-oxalic acid systems, presenting similar gelation conditions.

## Materials and methods

Chitosan ($\overline{M}_w = 2 \times 10^6$ g/mol; polydispersity = 3.86) was purchased from Marinard Biotech (Rivière-aux-Renards, QC). The DDA was estimated to be 90% by infrared spectroscopy from the absorbance ratio ($A_{1550}/A_{2878}$), considering baselines between 2000 and 1500 cm$^{-1}$ for the amide II stretching band, and between 4000 and 1850 cm$^{-1}$ for the 2878 cm$^{-1}$ reference band. The IR spectra were recorded on a BIO-RAD FTS 3000 infrared spectrophotometer (Mississauga, ON) from 0.25 mm thickness KBr pellets dried during 24 hours at 60$^{\circ}$C. Phosphoric and oxalic acids, supplied by Sigma-Aldrich (Oakville, ON), were used without further purification. Urea, an intermolecular hydrogen bond disrupting agent, was purchased from the same company and added at concentrations between 0.2 and 1M to selected chitosan solutions.

### Preparation of chitosan hydrogels

Phosphoric and oxalic acid aqueous solutions at different concentrations (0.3 to 1 M and 1.5 M, respectively) were prepared. Within the range of 1-6% w/v (g/100mL), chitosan was dissolved in each medium. Solubilization was achieved in 100mL beakers by heating in a hot water bath (80°C). To avoid air bubbles in the sample, which might affect subsequent rheological measurements, the samples were centrifuged (3700 RPM, 10 minutes) at 25°C, poured in disk-shaped moulds and subsequently stored in a cold room at 10°C to allow gelation. This temperature was chosen in order to accelerate gelation (it was also achievable at room temperature, but was taking much longer) and in order to avoid ice-forming in the hydrogels. The pH was measured at room temperature (25°C) in viscous solutions with an electrode for colloids (Thermo Orion electrode, model 9272; VWR, Ville-Mont-Royal, QC) and on gels surfaces with a flat electrode (Thermo Orion electrode, model 8135; VWR, Ville-Mont-Royal, QC).

### Rheological measurements

A controlled stress rheometer, AR-1000N from TA-Instruments (New Castle, DE), was used to measure the dynamic properties of the systems. Parallel plates of 60 mm diameter with smooth surfaces were used for the viscous systems, while 40 mm diameter parallel plates with rough surfaces (with approximately 400 µm roughness), were employed for the gels to prevent slip. The molded gels of 2.5 mm thickness and in the shape of disks were directly placed between the parallel plates. The measurements were carried out by applying frequency sweeps from 0.063 to 570 rad.s$^{-1}$ in the linear viscoelastic regime at 20 ± 0.2°C under the following conditions: 0.1 strain for liquid systems, and a stress of 50 Pa for solids. In order to avoid evaporation during measurements, the rheometer was enclosed in a polyester protective cache.

## Results and discussion

### Effect of polymer concentration

In Figure 1 we present the evolution of the complex viscosity ($\eta^*$) at a constant low frequency (0.1 rad/s) as a function of polymer concentration on a double-logarithmic scale. The break point $C_{gel}$ corresponds to a value between 4 and 5% w/v for both acids. In the first region ($C < C_{gel}$), the zero-shear viscosity followed a typical scaling:

$$\eta_{0\ phosphoric} \sim C^{3.1} \qquad\qquad (1)$$
$$\eta_{0\ oxalic} \sim C^{3.4} \qquad\qquad (2)$$

Surprisingly for such an associative polymer, the exponents of the zero-shear viscosity (3.1 and 3.4) are similar to that observed for most random-coil polysaccharides and neutral polymers, i.e. 3.4. In the gelation concentration regime ($C > C_{gel}$) the slopes increase considerably. In that region, the intermolecular interactions become increasingly important and finally cause a transition into the gel state. Polarizing optical microscopy revealed a large difference between entangled solutions and

gels. Both chitosan-oxalic acid and chitosan-phosphoric acid gels displayed birefringence at high polymer concentrations, while entangled solutions below $C_{gel}$ displayed isotropy, confirming the absence of organized structures (results not shown).
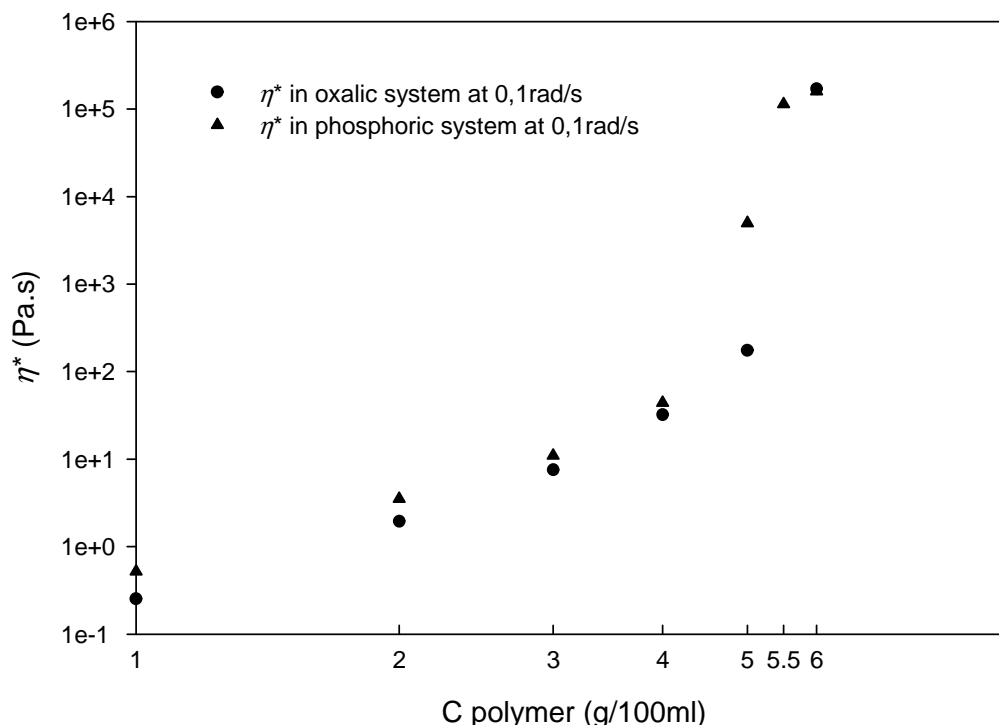


**Figure 1 :**   Complex viscosity at 0.1 rad/s and 20°C as a function of polymer concentration.


*Gelation kinetics*

In order to follow the kinetics of gelation in isothermal conditions, time sweep tests under small amplitude oscillatory shear were conducted at 10°C for both chitosan-acid solutions. Figure 2 shows the increase of the complex moduli as a function of time for the chitosan-oxalic acid system. A similar behavior was observed with phosphoric acid. Gelation seemed to occur in three stages: 1) a latent stage where *G'* and *G"* were almost constant with *G"* larger than *G'*, representing gelation incubation; 2) a rapid increase in *G'* past the crossover point (gel point), indicating a structure assembling; and 3) once the gel was formed, *G'* continued to evolve but more slowly due to the diffusion controlled motion of the molecules caused by the viscosity increase. In this last stage, the gel network rearranged slowly into more stable conformations to reach equilibrium. Tosh et al. [5] reported that two processes can occur concurrently in gelatin systems, which are temperature dependent systems as chitosan gels. The first one is a process of aggregation of individual polymers, which dominates during the early stage of gelation. The second step is the restructuring of the gel network into more energetically favorable conformations. Once the initial aggregation has been completed, the major contribution to the evolution of *G'* is the rearrangement of the polymer interactions which conducts the clusters to pack more closely.
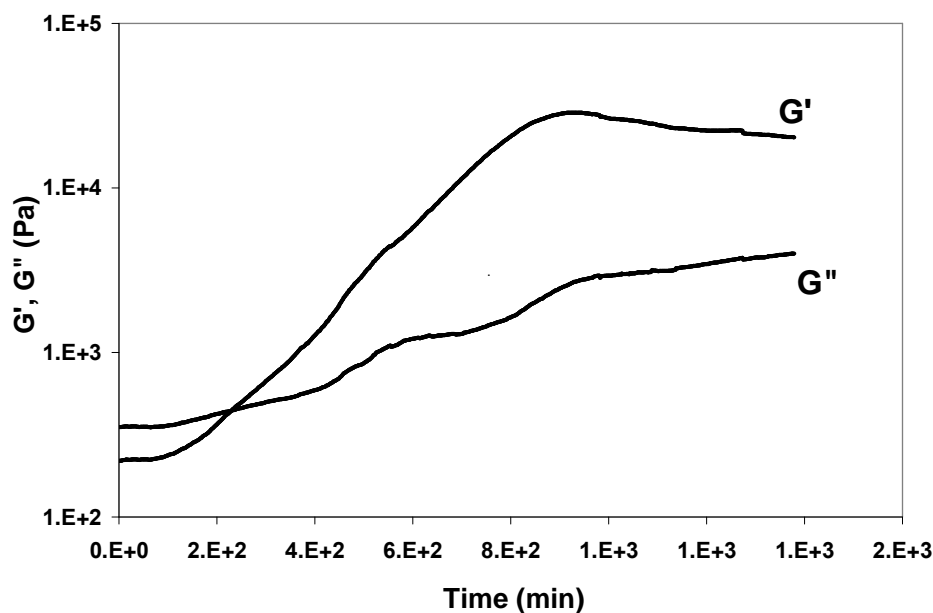
**Figure 2 :**    Gelation kinetics in 5% (w/v) chitosan-1M oxalic acid system. Complex moduli measured at 10°C, 6.28 rad/s and 0.1 strain.

Because of the critical nature of the sol-gel transition, it was difficult to probe dynamically the various samples exactly at the gel point. We conducted frequency sweep experiments at regular time intervals during the time sweep at 10°C in order to record information at different stages of the gel evolution (Figure 3). Tests at very low frequency were avoided (i.e. < 0.628 rad/s) in order to achieve dynamic measurements on a nearly constant structure. Frequency sweeps ran in the vicinity of the gel point for the oxalic acid system showed the quasi-superposition of $G'$ and $G''$ (Figure 3, insert at the crossover point). It should be mentioned that the approximate gel time $t_{gel}$ (time at which $G'$ crosses $G''$) varied from one sample to another ($t_{gel} = 5 \pm 0.8$ h), most probably because of the transient nature of the non-covalent junctions.
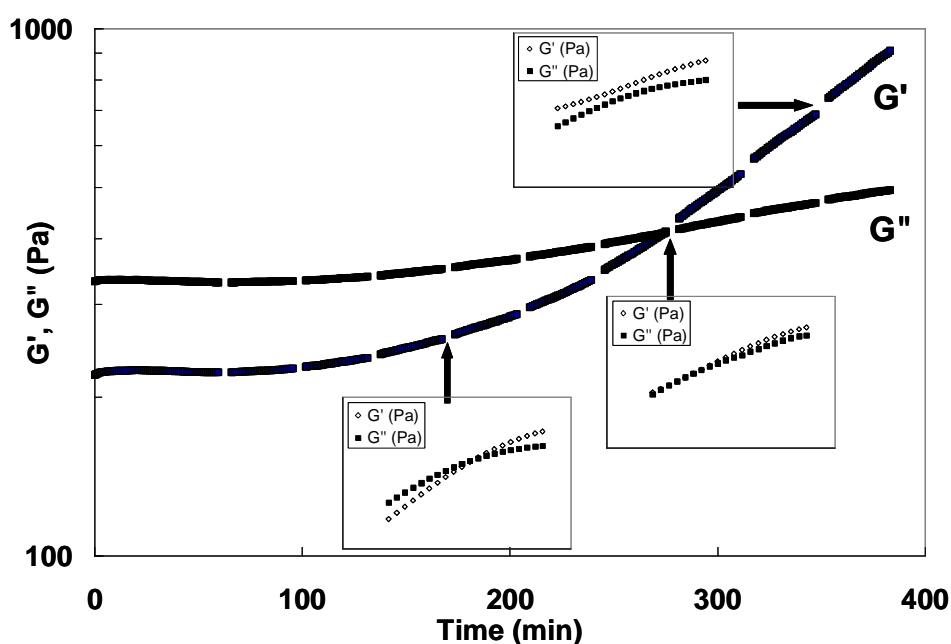


**Figure 3 :**    Results of frequency sweeps superposed on a time sweep at 10°C, before, at and beyond the gel point for a 5% (w/v) chitosan-1M oxalic acid solution. Frequency sweeps (0.63-314 rad/s) ran at 0.1 strain.

*Thermoreversibility*

Thermoreversibility was investigated by applying several cooling-heating cycles on molten gels as shown in Figure 4 for the chitosan-phosphoric acid system ($\gamma_0 = 0.1$, $\omega = 6.28$ rad/s, $dT/dt = 3°C/min$). During the first cooling-heating cycle both systems recovered their initial state (results for oxalic acid not shown). However, when further cycling was applied on the same gels they gradually lost their thermoreversibility and became permanent. The kinetics of gel formation also became faster, as reported by Hayes and Davies [3] for the chitosan-oxalic acid system. We observed that this system became permanent at the second cycle, while the phosphoric acid system could be solidified and molten two more times, revealing a higher thermoreversibility. These results gave an indication that the physical interactions making the gels became stronger as thermal energy was added, to the point of showing a permanent character as for chemical gels. It was also noticed that the mechanical properties of the oxalic acid gels in the first cycle were much larger than those of the phosphoric acid one even at the third cycle. Its apparent activation energy for flow (265 kJ/mol) was also higher than that of the phosphoric acid-based system (202 kJ/mol) ([1]), slightly closer to that of chemical gels (~ 400 kJ/mol) ([6]). The loss of the thermoreversible character may be explained by the presence of di-anions in the gel network. Upon heating to 80°C, the presence of divalent species becomes larger due to the decrease of the dissociation constants $K_a$, and the divalent species can be up to 4% for the oxalic acid. We have hypothesized that rapid successive cooling and heating cycles may inhibit some di-anions confined in the physical network (Figure 5) to go back to their monovalent dissociated state, giving therefore a strong ionically cross-linked character to the systems. Further investigation using Fourier-Transform infra-red spectroscopy (FTIR) may help us infer the presence of permanent oxalic or phosphoric acid anions in the chitosan gel structures.
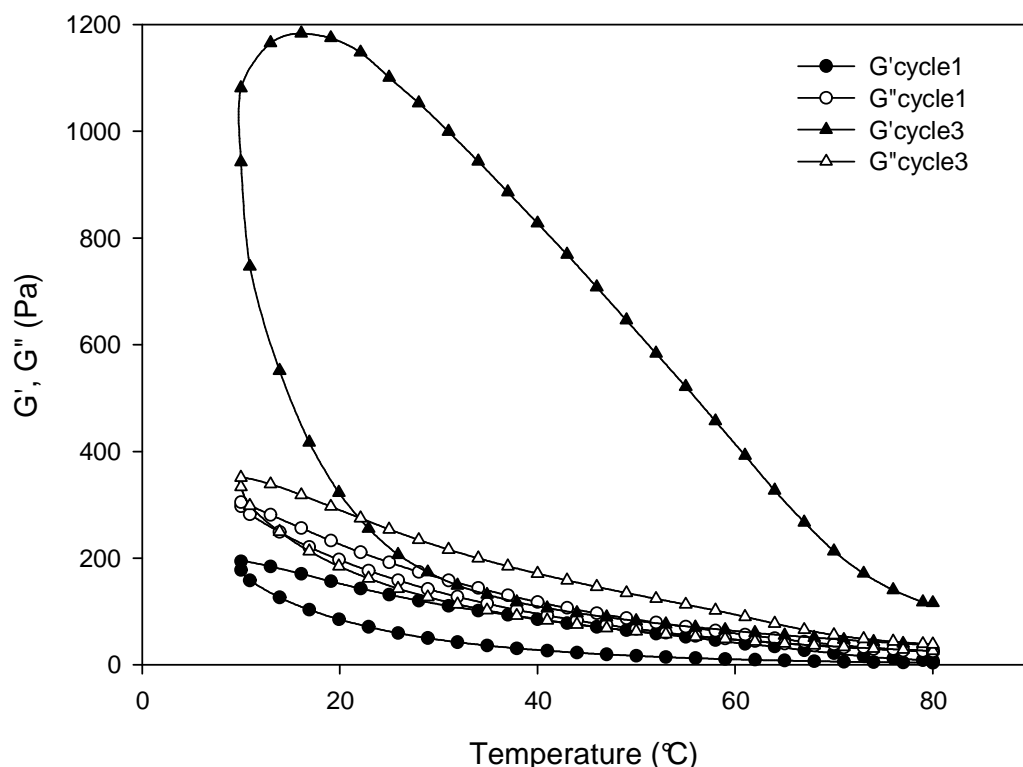


**Figure 4 :** Evolution of complex moduli of chitosan-phosphoric acid systems in successive coolings (1) and heatings (2).
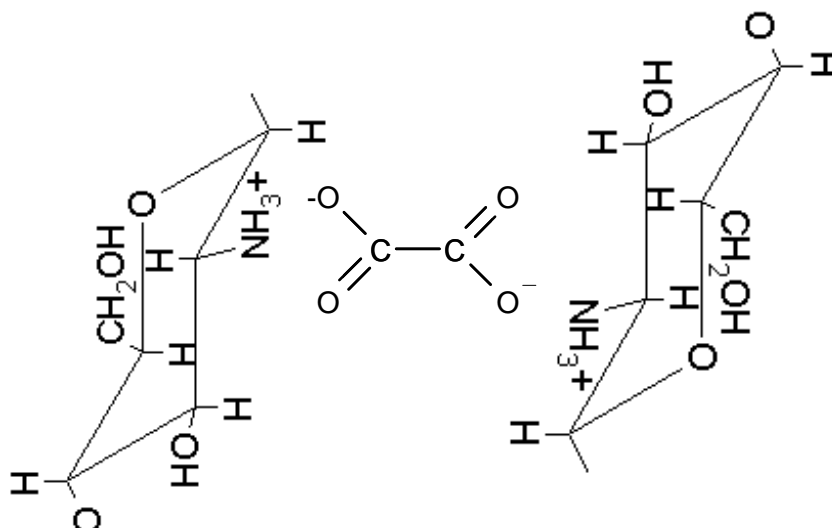
**Figure 5 :**    Oxalic acid di-cations ionically bridging two chitosan glucosamine units.

## Conclusions

In this work, the concentration induced gelation of chitosan-phosphoric acid and chitosan-oxalic acid systems was investigated. Dynamic rheological measurements revealed that gelation required high polymer concentration to occur, i.e. around 5% w/v.

Kinetic studies revealed that the gelation occurred in three stages: 1) an incubation stage with an almost stable $G'$ and $G''$ up to the gel point; 2) a fast stage with a rapid increase of $G'$, indicating an increase in the number of long chains and a structure assembling; 3) and a slow stage where $G'$ continued to evolve slowly and reached equilibrium through gel network rearrangement into more stable conformations.

Successive cooling-heating cycles applied on molten gels within the range of 80-10°C showed that the systems recovered their initial state during the first cycle, and therefore thermoreversibility. However, in subsequent cycles the gels became permanent, possibly due to increased ionic cross-linking by divalent anions species.

## Acknowledgements

## References

**[1]**   M. Hamdine, M.C. Heuzey, A. Bégin, Int J. Biol Macromol, 37(2005) 134-142.

**[2]**   M. Hamdine, M.C. Heuzey, A. Bégin, Rheol. Acta, 45(2006), 659-675.

**[3]**   E.R. Hayes, D.H. Davies, Proceedings of the First International Conference on Chitin/Chitosan. Eds. R.A.A. Muzzareli, E.R. Parsier, Cambridge (1978), 193-197.

**[4]**   R. Yamaguchi, S. Hirano, Y. Arai, T. Ito, Agr. Biol. Chem, 42(1978), 1981-1982.

**[5]**   S.M. Tosh, A.G. Marangoni, F.R. Hallet, I.J. Britt, Food Hydrocolloids 17(2003), 503-513.

**[6]**   A.H. Clark, S.B. Ross-Murphy, Adv. Polymer Science 83(1987), 57-184.