

## Rheological Behaviour of Mixed Biopolymer Systems Undergoing Order/Disorder Transitions

Kurt Ingar Draget, Frode Magnussen, Kirsti Hedalen, Olav Smidsrød and Ingvild Haug

Norwegian Biopolymer Laboratory  
Dept. of Biotechnology  
Norwegian University of Science and Technology  
N-7491 Trondheim, Norway

### ABSTRACT

Properties of mixed gels made from two different thermo-setting biopolymers at associative phase separated conditions are presented as function of temperature. Special emphasis is put on the relative importance of the two polymers in relation to network formation and apparent equilibrium modulus. The mechanical properties are linked to differences in turbidity, being an indirect measure on the presence of light scattering aggregates

### INTRODUCTION

Gelatin from cold water fish (FG) and  $\kappa$ -carrageenan (CG) are two biopolymers with distinct and different gelling temperatures, being typically 8 and 30 °C, respectively<sup>1, 2, 3</sup>. The reason why gelatins from cold water fish species differ significantly from gelatins from mammalian origin (e.g. bovine and porcine) with respect to setting and melting temperature is the relative low amounts of imino acids (proline and hydroxyproline) which are known to stabilise the ordered triple helix<sup>3</sup>. By adjusting parameters like pH and ionic strength, these poly-ions can also associatively interact through electrostatic bridging. And by exploiting the properties of fish gelatins rather than mammalian ones, an opportunity arises where the network formation and interactions can be studied as the two poly-electrolytes undergo well (temperature-) separated order/disorder transitions.

The scope of this paper was to evaluate, and if possible to quantify, the macroscopic rheological changes taking place in such mixed systems when lowering the temperature from 60 °C, via 20 °C to 4 °C. On a molecular scale, this corresponds to both poly-ions being in a random coil conformation and associatively phase separated (60 °C),  $\kappa$ -CG being ordered and FG being disordered (20 °C) and both polymers being in an ordered helix conformation (4 °C).

### MATERIALS AND METHODS

The cold water fish gelatin sample used in this study was kindly provided by Norland Products Inc., and was manufactured by hydrolysing collagen from fish skins. The weight average molecular weight was measured by SEC-MALLS to be  $170 \pm 17$  kDa. Isoelectric point (IEP) was measured by electrophoresis and found to be 8.7.

The  $\kappa$ -carrageenan sample was kindly provided by FMC Biopolymer, and was 91% pure with the remaining 9% being  $\iota$ -carrageenan. Weight average molecular weight was found to be 630 kDa by SEC-MALLS. The residual amount of  $K^+$  was measured by atomic absorption, and was found to be equivalent to 14 mM in a 10 mg/ml solution.

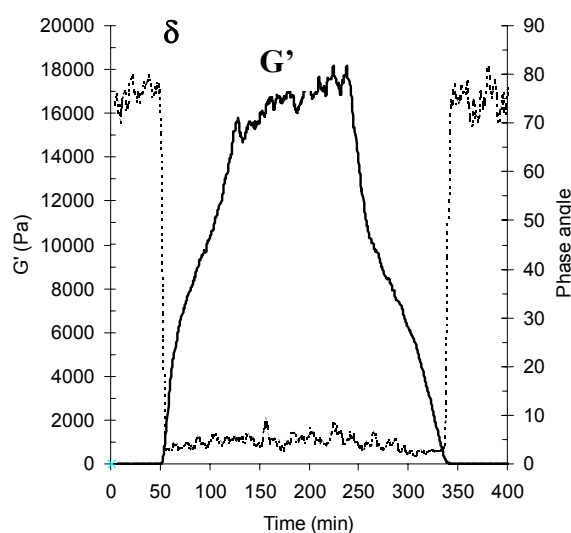
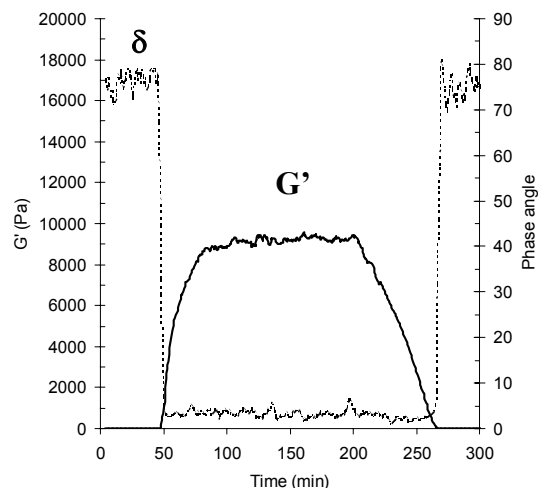


Figure 1. Sol-gel transition of a mixed system consisting of 2% FG and 1% CG with 20mM added  $K^+$  going from 60 to 20°C (top) and from 60 to 4°C (bottom)

The mixed gels were prepared as follows: A solution of double concentrated CG (relative to the final gel concentration) was prepared by heating a sealed container under stirring for 30 minutes at 90°C, and placed in an incubator at 60°C for 30 minutes. The double concentrated FG solution was made by dissolving at room temperature, and placed in the same 60 °C incubator for 30 minutes. Whenever added, additional KCl was mixed in the CG

solution. The two solutions were then mixed under stirring, and quickly poured into pre-formed wells with dimensions of 18 (h) times 16 (d) millimetres. Samples at 4 °C and 20°C were taken out for compression analysis after 18 hours by applying a Stable Micro Systems TA.XT2 texture analyser at a compression speed of 0.1 mm s<sup>-1</sup>. Young's modulus was calculated from the initial part of the resulting force-deformation curve<sup>4</sup>.

Some mixed samples at 60°C were also placed between a serrated plate-plate measuring geometry (gap = 1 mm) in a Reologica StressTech general purpose rheometer to follow gelling kinetics during lowering of the temperature. Measuring parameters: temperature gradient of 0.5°C min<sup>-1</sup>, different stresses, and a frequency of 1 Hz.

Turbidity was measured on the mixed gels by applying a Bio-Rad Novopath Mini Reader at 405 nm.

## RESULTS AND DISCUSSION

Figures 1a and b show typical sol/gel transitions of a mixed system of FG and CG starting from an associatively phase separated system at 60°C and cooling down to 22°C (1a) and 4°C (1b). After starting

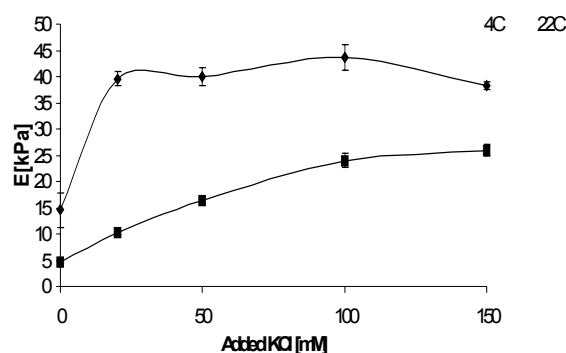


Figure 2. Apparent equilibrium Young's modulus (E) for a mixed system consisting of 10% FG and 0.8% CG at different  $K^+$  concentrations at 20 and 4°C

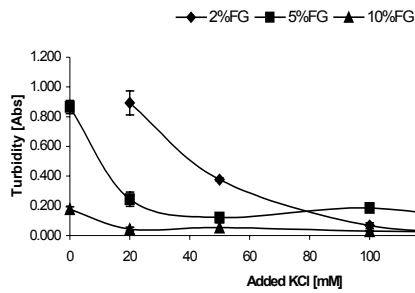


Figure 4. Turbidity as function of FG and  $K^+$  concentration at 0.8% CG and 4°C

from a turbid, almost colloidal liquid dispersion at this temperature, it is readily seen that there are two distinct setting regimes (Figure 1b). These setting temperatures correspond to the inherent properties of the two components alone; CG gelling at around 30°C and FG below 10°C. It can also be observed that the last setting, being governed by the properties of FG, represents the highest increase in elastic properties (most pronounced increase in the dynamic storage modulus ( $G'$ )). This is rather unexpected since the 2% FG alone can not account for this increase ( $G'$  at 4°C in the vicinity of 10 Pa; results not included). Lowering from 22°C to 4°C does result in an increased  $G'$  for the carrageenan gel alone, but not to the same extent as shown in Figure 1b. Additionally, during holding-time at 4 °C one can almost spot the fingerprint of a non-equilibrium gelatin gel ( $G'$  increases substantially with time). These results suggest that FG certainly participate in the build-up of elastic properties at low temperature, and that some sort of interaction between the two types of polymers may be present even when both are in the ordered conformation.

Figure 2 shows the apparent equilibrium Young's modulus ( $E$ ) in a mixed system of FG and CG at 20 and 4°C, and with varying concentrations of  $K^+$ . Again, a most pronounced increase in elastic properties going from 20 to 4°C is observed; especially at low  $K^+$  additions. Practical limitations make it difficult to measure  $E$  for a pure

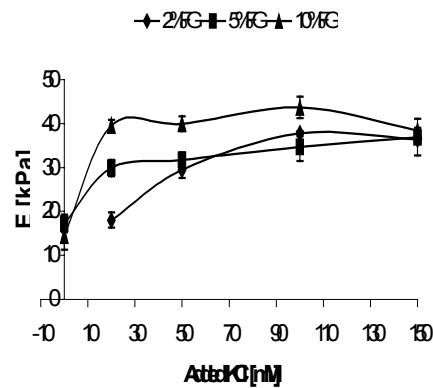
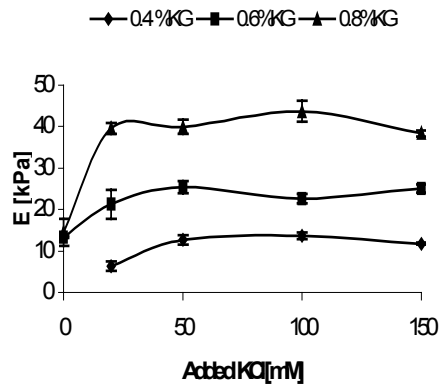


Figure 3. The change in the apparent equilibrium Young's modulus with varying amount of FG at 0.8% CG(A) and varying CG at 10% FG (B) at 4°C

10% FG gel at 4°C, but the dynamic shear modulus at these conditions is 900 Pa. Adapting an ideal elastic behaviour (Poisson's ratio  $\mu$  equals 0.5) suggests that the Young's modulus should be close to 3 kPa<sup>5</sup>. Anyway, the contribution from the FG itself can not under any circumstances account for the e.g. 30 kPa increase at 20 mM addition of  $K^+$ . The increase in  $E$  for the pure 0.8% CG going from 20 to 4°C is 10-15 kPa (results not included), suggesting that the increase in  $E$  for the mixed system is somewhat higher than can be expected from the two components alone.

The observation that the FG component plays a minor role in this mixed system compared to its CG counterpart is

strengthened by the results presented in Figures 3 a and b. These figures demonstrate

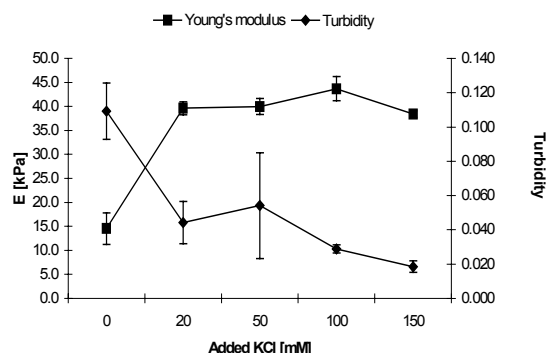


Figure 5. Young's modulus and turbidity as function as added KCl of 10% FG and 0.8% CG at 4°C

the change in the apparent equilibrium Young's modulus with varying amount of FG (Fig. 3a) and varying amount of CG (Fig. 3b). Figure 3a indicates some differences in elastic properties at low (< 50 mM) amounts of added  $K^+$ , but that these differences diminish at higher  $K^+$  concentrations and finally disappear. In the case of varying the amount of CG, there is a pronounced and lasting increase in  $E$  with increasing CG concentration at all levels of  $K^+$ . A closer examination of Figure 3b results in a very good correlation between Young's modulus and CG concentration in the 50-100 mM  $K^+$  regime if one adapts the general  $E \propto c^2$ <sup>7, 8</sup>. All in all, these results suggest that CG is the governing component of the elastic properties of these mixed gels at 4°C, even though it is the minor component with respect to relative amount and even though both polymers are in the ordered conformation.

The turbidity of such mixed systems, which indirectly quantify the relative amounts of light scattering aggregates, is presented in Figure 4 as function of varying amounts of FG and  $K^+$ . It contrast to varying the amount of CG, which in the present regime did not alter the measured turbidity to a great extent (results not included), a profound effect of FG concentration on

turbidity is observed. This is valid for  $K^+$  concentrations from 50 mM and below. The increased turbidity in this regime with decreasing FG content is likely to be related to the growth of aggregates towards electroneutrality<sup>9,10</sup> as the overall composition of the system becomes more electrostatically balanced to favour this situation. At high  $K^+$  concentrations, there are hardly any difference in turbidity between gels of different FG content. This is most likely due to a combination of non-specific ionic strength effects (shielding off of electrostatic charges and a reduced entropic drive for associative phase separation<sup>11</sup>), and a specific  $K^+$  effect favouring CG to associate into a simple and homogeneous CG network rather than supporting complex CG-FG interactions.

The reduced degree of turbidity at low (< 50 mM)  $K^+$  concentration and high (e.g. 10%) FG content is most prone to be due to a system being disproportionate with respect to electrostatic charges. This situation will inevitably lead to the formation of soluble complexes rather than insoluble, electroneutral complexes; the former hardly scattering any light compared to the latter<sup>9</sup>. Even though Figure 4 shows almost no turbidity in the system containing 10% FG, some exciting results still pop up when these values are compared with the apparent equilibrium Young's modulus (Figure 5). This figure displays that even minute changes in turbidity point toward extensive differences in the elastic properties. Again, this result suggest that a reduced degree of phase separation (less turbidity) results in gels with increased mechanical properties, probably due to a higher volume fraction of homogeneous carrageenan gels.

## CONCLUSIONS

An initially associatively phase separated system can, at least partially, be reversed into more single phased (i.e. pure CG and pure FG) combinations by forcing the components to undergo disorder/order transitions. The overall elastic properties of

the present system can not, however, be directly explained by the contribution of the single components alone. The remaining question, therefore, is if the net increase in the mechanical properties is due to a simple excluded volume effect, an effect of FG as purely a filler in a continuous CG network, or if what might be called a “coupled, filled network” still is present at 4°C.

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