

Rheological properties of gels formed with furcellaran and globular proteins bovine serum albumin and β -lactoglobulin

Katrin Laos¹, Geoffrey J. Brownsey², Margus Friedenthal¹ and Stephen G. Ring²

¹Department of Food Processing, Tallinn University of Technology, Ehitajate tee 5, Tallinn 19086, Estonia

²Division of Food Materials Science, Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, UK

ABSTRACT

The effect of the addition of the globular proteins, bovine serum albumin and β -lactoglobulin, on the properties of furcellaran gels was studied. The gels were found to be physically cross-linked networked gels. Proteins strongly influence the shear modulus. At low pH the phase-separation occurred with the change in loss tangent.

INTRODUCTION

Both proteins and polysaccharides contribute to the structural properties of foods through their aggregation and gelation behaviour. The development of novel gelled products from proteins and polysaccharides and their control needs a better understanding of gelation mechanisms and physical properties of mixed gels¹.

When gelation occurs between two different polymers, three basic types of gel structure can be observed, namely interpenetrating, associative, and phase-separated networks. Interpenetrating networks form when the two components gel separately and form independent networks. Both networks are continuous throughout the sample but any interaction between them is only topological². Associative networks are formed when there are direct associations between polymers

prior to network formation. Phase-separation can be segregative (also called thermodynamic incompatibility) and associative (also called complex coacervation). In associative phase separation one of the separating phases is enriched in both polymers, whilst, in a segregative phase separation each phase is enriched in one of the polymers³. When phase separation takes place, a competition between the phase separation and gelation process generally takes place, resulting in an increase in the complexity of the system⁴.

The understanding of the mechanisms of interaction and the effect of changing conformation on these interactions is of great interest for predicting the structure and the properties of mixed systems⁵.

Furcellaran is an anionic sulphated polysaccharide extracted from the red alga *Furcellaria lumbricalis*. It is currently considered to be a copolymer of β - and κ -carrageenan and usually represented structurally as a repeating unit of alternating 3-linked β -D-galactopyranose and 4-linked α -D-galactopyranose residues, with part of the latter existing as a 3,6-anhydro derivative⁶ (Figure 1). Hydroxyl groups in the polysaccharide chain may be substituted (sulphated, methylated, etc.) and other monomer residues such as xylose and glucose may be found⁷. Furcellaran is a

polyelectrolyte that carries sulphate groups and it is negatively charged over a wide range of pH. The charge density is usually one sulphate per three or four monomer units⁸.

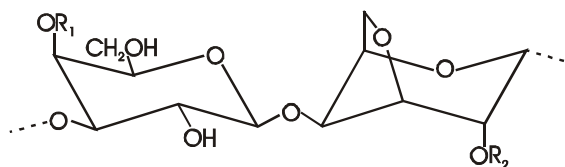


Figure 1. Primary structure formulae of the repeating disaccharide unit of β -($R_1=R_2=H$) and κ -($R_1=OSO_3^-$, $R_2=H$)

A characteristic of furcellaran is its ability to form gels in the presence of specific ions which is associated with a conformational change from coil to helix. Gelation of furcellaran is believed to proceed like κ -carrageenan via a two-step mechanism, as shown in Figure 2. Carrageenan chains associate themselves by the formation of intermolecular double helices, but these do not in themselves produce a gel network. Gelation occurs with the subsequent aggregation of these helices mediated by specific binding of gel promoting cations (particularly K^+ and Ca^{2+})⁹.

Bovine serum albumin (BSA) is one of the most widely studied and applied proteins in biochemistry. It is an important, globular blood protein with molecular mass of 66,000. Serum albumins comprise about 580 amino acid residues and are characterized by a low content of tryptophan and a high content of cystine and charged amino acids, such as aspartic and glutamic acids, lysine and arginine¹⁰.

β -lactoglobulin (BLG) is a major protein of the whey fraction of milk in many mammals. At physiological conditions, bovine β -lactoglobulin forms dimer, with each monomer consisting of 162 amino acid residues and characterized by a molecular mass 18,300. The conformation of BLG is pH dependent. At pH 3.5 the protein

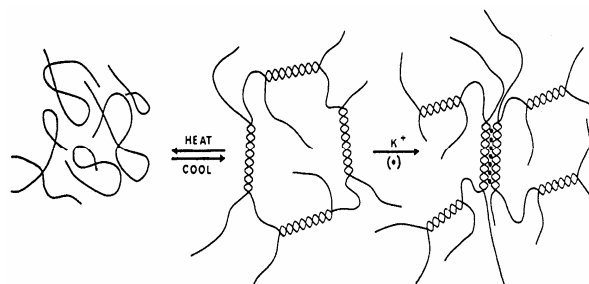


Figure 2. The two-step “domain” model for gelation of κ -carrageenan⁹.

dimerizes, at pH 4.6 the dimers may further aggregate to form an octamer and at pH 7.5 the protein conformation undergoes a reversible transition which leads to a swelling of the monomeric unit¹¹.

The aim of this study is to investigate the rheological behaviour of mixed gels formed with furcellaran and globular proteins, BSA and BLG.

EXPERIMENTAL

Materials

The furcellaran was a gift from FMC Food Ingredients.

BSA and BLG were supplied by Sigma-Aldrich. They were used without further purification. The potassium acetate and potassium phosphate buffer solutions were prepared with analytical grade reagents.

Purification of furcellaran

The furcellaran preparation (3g) was dissolved in 2 L milliQ water at $\sim 100^\circ\text{C}$. The solution was passed through diatomaceous earth (Sigma-Aldrich Co, Ltd., UK) and dialyzed (yield 80%). The Na^+ furcellaran was obtained by elution through an ion-exchange (Amberlite IR-120) column in the Na^+ form at 4°C . The eluant was freeze-dried.

Preparation of gels

Protein and furcellaran samples were dispersed separately in water or buffer solutions and then mixed. The gels were

prepared by dialysing the solutions against 1M KCl solution in water or buffer solution at 4°C for overnight.

Rheometry

The stiffness of the gels at 25°C was determined as the shear modulus at 200 Hz calculated from the measured velocity of a shear-wave passing through the gel using a Rank pulse shearometer¹².

The rheological properties of the gels were analysed by dynamic oscillatory controlled strain measurements using the Advanced Rheometric Expansion System (ARES-LS2). The viscoelastic properties of the gels were analysed by recording the storage modulus (G'), loss modulus (G''), and loss tangent ($\tan\delta$) during strain and frequency sweeps at 25°C. A parallel plate measuring geometry was used with gel diameter 4mm. The gel pieces were carefully placed on the lower plate of the rheometer, and a compression of not more than 0.5% was applied to the gel by the upper plate. Strain sweep measurements were performed at a constant frequency of 1 Hz whilst frequency measurements were performed using 0.1% strain, which was within the linear viscoelastic region for furcellaran and mixed gels.

RESULTS AND DISCUSSION

The dependence of shear modulus on the furcellaran concentration is shown in Figure 3. The shear modulus increased with increasing concentration of furcellaran. In Figure 4 is shown dependence of shear modulus on the KCl concentration (0.1-1M) for the 1.5% furcellaran gel. The shear modulus increased with KCl concentration, and was more marked in the concentration range 0 to 0.4 M KCl.

Dynamic oscillatory rheology was used to monitor more detailed gel characteristics. To ensure that the gel properties were measured within the linear viscoelastic region, strain sweep measurement were first carried out (Figure 5). It can be seen that

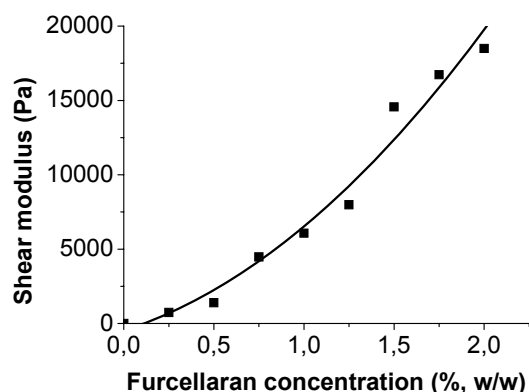


Fig.3. Dependence of shear modulus on furcellaran concentration.

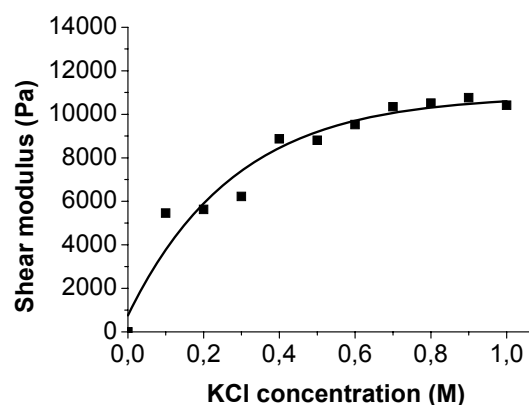


Fig.4. Dependence of shear modulus on KCl concentration

of 1% (1.5% furcellaran gel) and 0.8% (mixed gel) the gel characteristics start to change. This is caused by gel slipping between the plates of the rheometer and can be explained by syneresis. The same behaviour had been observed before also with κ -carrageenan gels^{9,13,14} and is said to be a limiting factor in the measurements of the mechanical properties of carrageenan gels. In our case the syneresis was more marked gels with low pH. For all samples the linear viscoelastic region was below a strain of 0.5%. Thus for frequency sweep measurements a strain value 0.1% was chosen.

The furcellaran and mixed gels in the studied pH range, showed a frequency

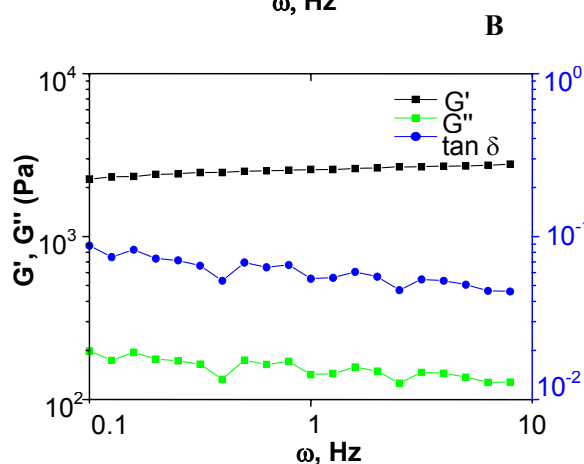
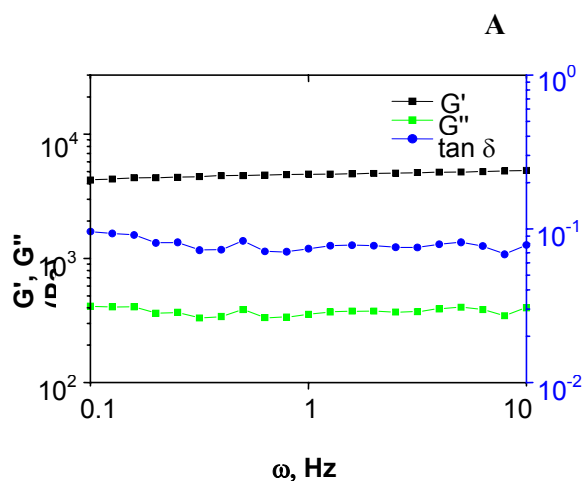


Figure 5. Shear modulus (G'), loss modulus (G'') and loss tangent ($\tan \delta$) as a function of strain for furcellaran (1.5% w/w) (A) and furcellaran (1.5% w/w) -BSA (0.5% w/w) mixture (B).

dependence of G' but no $G'-G''$ crossover and can be categorised as a typical physically cross-linked network gel (Figure 6). A physical gel is a polymer network in which junctions can break and recombine due to thermal fluctuation. The variation of storage modulus and loss modulus was small with added protein (Figure 6B) or changing pH.

For the shear modulus in mixed furcellaran/protein gels, complex behaviour was observed. The initial addition of 0.1% w/w protein to the 1.5% furcellaran solution increased shear modulus, further increase in protein content lead to a decrease in gel

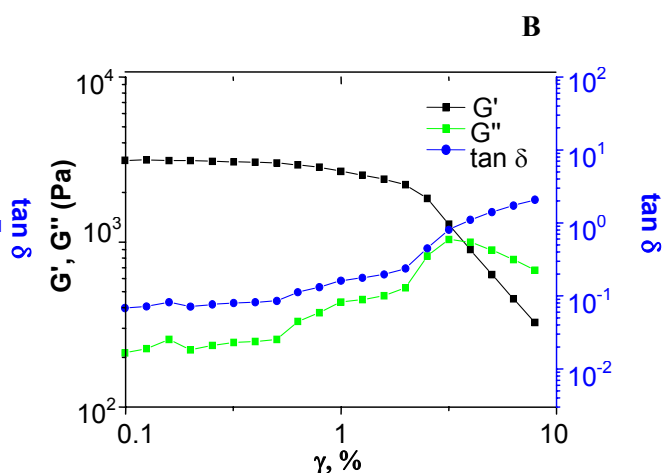
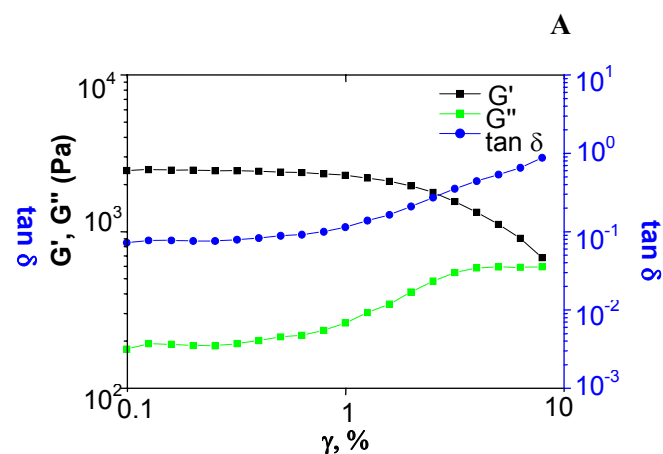


Figure 6. Shear modulus (G') and loss modulus (G'') as a function of frequency for furcellaran (1.5% w/w) (A) and furcellaran (1.5% w/w) -BSA (0.5% w/w) mixture (B).

stiffness (Figure 7). In the range 0.5% to 2.5% (w/w) BSA and 0.75 to 2.5% (w/w) BLG, increase in modulus was observed. From a protein content of 2.5% (w/w) the gel stiffness decreased again. Similar behaviour in G' was recently reported by Oakenfull et al.⁹ with gels made with κ -carrageenan and sodium caseinate. Considering their work, the behaviour between furcellaran and protein can be explained as follows:

1. When small amounts of protein are added, the dry matter content is increased and the G' increases.

- When more protein is added, some of the furcellaran is bound to the protein. Bound furcellaran is no longer available to contribute to the furcellaran network. Thus G' decreases.
- As the concentration of protein increases, protein aggregates with bound furcellaran become sufficiently abundant to form a continuous network, G' then increases.

In figure 7 (dotted curves) is shown the separate contributions to G' from the complex and furcellaran. The contribution of furcellaran decreases, as with increasing concentration of protein the furcellaran forms the complex with protein; the contribution from the complex increases, once it has passed the gel threshold.

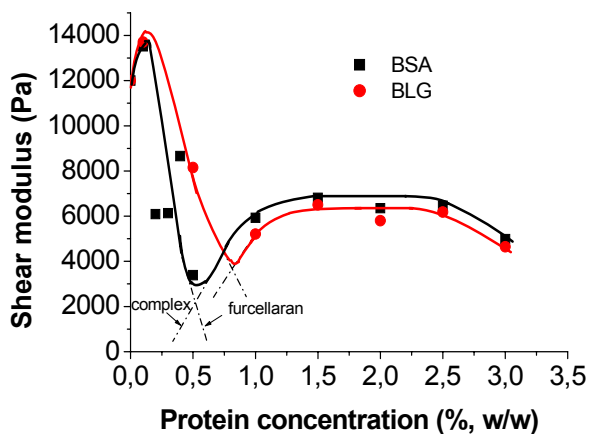


Figure 7. Dependence of shear modulus, G' 1 Hz, on BSA or BLG concentration in 1.5% w/w furcellaran gels.

Studying the dependence of pH on the gels, we observed that the structure of the mixed furcellaran/protein gels was pH dependent. At pH 7 the gels were translucent but became turbid with decreasing pH (Figure 8). This change indicates a phase separation between furcellaran and the protein. Looking the furcellaran/BSA mixed gel at pH=3.4 under the microscope we can see a coarse, phase separated structure (Figure 9).

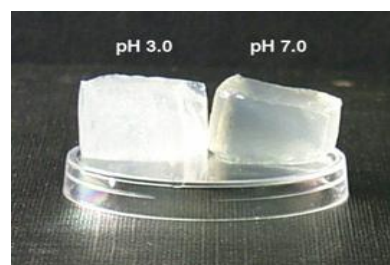


Figure 8. 1.5% w/w furcellaran gels with 1.5% w/w BSA at different pH's.

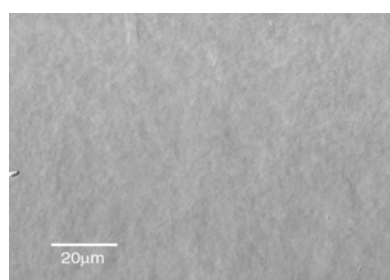


Figure 9. Optical micrograph of a 1.5% w/w furcellaran gel containing 1.5% w/w BSA at pH 3.

The dependence of shear modulus on the pH of furcellarans gels is shown in Figure 10. The shear modulus is increasing with increasing pH of medium, goes through maximum at pH 4.3 and begins to decrease. The gel stiffness starts to increase again from pH 6.2. The mixed systems behave in a similar way but the shear modulus values are higher. This behaviour indicates that low pH increases the stiffness of the gel but from pH 4.3 it starts to decrease again.

As in these measurements there wasn't a marked behaviour difference between furcellaran and furcellaran/protein gels, dynamic oscillatory rheology was applied. In Figure 11 it can be seen that for furcellaran gels the loss tangent increased linearly with increasing pH. But for mixed systems there was decrease in loss tangent between pH 3.4 and 4. From pH 4 the loss tangent increased linearly with increasing pH.

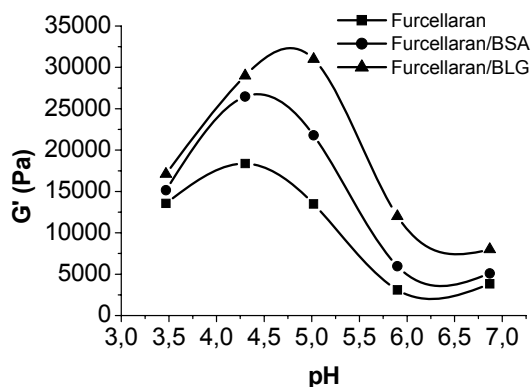


Fig.10. Dependence of shear modulus G' at 0.1 Hz on the pH of gels formed from 1.5% (w/w) furcellarans and 1.5% furcellaran and 1.5% protein (w/w) mixed gels.

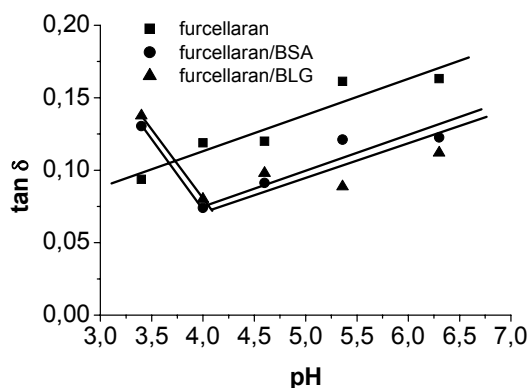


Fig.11. Dependence of loss tangent $\tan \delta$ at 0.1 Hz on the pH of gels formed 1.5% w/w furcellarans and 1.5% furcellaran and 1.5% protein (w/w) mixed gels.

The results obtained show that when gelation occurs in furcellaran globular protein systems, at pH's in the vicinity of neutrality, an associative network forms. On decreasing the pH of the medium to pH 3.4 a phase separated structure forms.

CONCLUSIONS

This study shows that furcellaran and globular proteins form physically cross-linked network gels. The initial addition of protein to furcellaran solution increased shear modulus, further increase in BSA

content leads to a decrease in gel shear modulus. Studying the dependence of pH on the gels we observed that the structure of the mixed furcellaran/protein gels was pH dependent. At low pH the associated phase separation occurred and loss tangent changed.

ACKNOWLEDGEMENTS

The authors thank the BBSRC core strategic grant for financial support; the EC Commission for the award of a Marie Curie fellowship to K.L. (Contract Number QLK-1999-50512)

REFERENCES

- Baeza, R., Gugliotta, L.M., Pilosof, A.M.R. (2003), "Gelation of β -lactoglobulin in the presence of propylene glycol alginate: kinetics and gel properties", *Colloids and Surfaces B: Biointerfaces*, **31**, 81-93.
- Morris, V. J. (1986). „Multicomponent gels.” In Philips G.O., Wedlock, D.J., Williams, P.A. (Eds.) *Gums and stabilisers for the food industry*, 3. Elsevier, London, pp. 87-99
- Piculell, L., Bergfeld, K., Nilsson, S. (1995). „Factors determining phase behaviour of multi-component polymer systems”. In Harding, S., Hill, S.E., Mitchell, J.R. (Eds.) *Biopolymer mixtures*, Nottingham University Press, Nottingham, pp. 13-35.
- Ould Eleya, M.M., Turgeon, S.L. (2000), "Rheology of κ -carrageenan and β -lactoglobulin mixed gels", *Food Hydrocolloids*, **14**, 29-40
- Michon, C., Konatè, K., Cuvelier, G., Launay, B. (2002), "Gelatin/carrageenan interactions in coil and ordered conformations followed by a methylene blue spectrophotometric method", *Food Hydrocolloids*, **16**, 613-618.
- Hugerth, A., Sundelöf, L.-O. (2001), "The effect of polyelectrolyte counterion specificity, charge density, and conformation on polyelectrolyte-amphiphile interaction: the carrageenan/ furcellaran -

- amitriptyline system”, *Biopolymers*, **58**, 186-159.
7. Van de Velde, F., Knutsen, S.H., Usov, A.I., Rollema, H.S., Cerezo, A.S. (2002), “¹H and ¹³C high resolution NMR spectroscopy of carrageenans: Application in research and industry”, *Trends in Food Science & Technology*, **13**, 73-92.
8. Glicksman, M. (1983), “Food Hydrocolloids”, CRC Press, New York, pp 83-107.
9. Oakenfull, D., Miyoshi, E., Nishinari, K., Scott, A. (1999), “Rheological and thermal properties of milk gels formed with κ-carrageenan. I. Sodium caseinate”, *Food Hydrocolloids*, **13**, 525-533
10. Peters, T. (1985), „Advances in Protein Chemistry”, vol. **37**, Academic Press, New York, pp. 161–245.
11. Verheul, M., Pedersen, J.S., Roefs, S.P.F.M., de Kruijff, K.G. (1999), “Association behaviour of native β-lactoglobulin”, *Biopolymers*, **49**, 11-20.
12. Ring, S.G., Stainsby, G. (1985), “A simple method for determining the shear modulus of food dispersions and gels”, *J. Sci. Food Agric.*, **36**, 607-613
13. Lai, V.M.F., Wong, P.A.-L., Lii, C.-Y. (2000), “Effects of Cation Properties on Sol-gel Transition and Gel Properties of κ-carrageenan”, *Journal of Food Science*, **65**, 1332-1337.
14. MacArtain, P., Jacquier, J.C., Dawson, K.A. (2003), “Physical Characteristics of Calcium Induced Kappa- Carrageenan Networks”, *Carbohydrate Polymers*, **53**, 395-400