

was increased by increasing the pH (the gelatin chains become less positively charged,  $pI = 9$ ) and/or by increasing the sodium ion concentration (screening of electrostatic interactions). Temperature did not greatly influence the size of the incompatible region. This is in agreement with the hypothesis that attractive electrostatic interactions lead to associative phase separation (traditionally called complex coacervation).

The influence of pH and sodium concentration was studied in more detail for two mixtures of 3% gelatin and 0.9% iota-carrageenan: g2/il and g3/il. Both pH, between 4 and 9, and sodium ion concentration, between 0 and 1.2 M, had significant effects on the phase behaviour.

#### SIMULTANEOUS PHASE SEPARATION AND GELATION IN AQUEOUS SYSTEMS OF DEXTRAN AND GELATINE

R.H. TROMP, A.R. RENNIE and A.L. JONES

*Polymer and Colloids Group, Cavendish Laboratory, University of Cambridge, Madingley Road, Cambridge CB3 0HE, UK*

The mutual influences of the gelation process and the kinetics of phase separation in an aqueous solution of dextran and gelatine have been studied. Below the phase transition temperature, the solution separates into dextran-rich and gelatine-rich phases. On further cooling, the gelatine causes the gelatine-rich phase to gel. Results of time resolved small angle static light scattering and phase contrast microscopy show that below a certain temperature nearly the entire phase separation process is influenced by the gelling tendency of the gelatine. The rate of gelation relative to that of the phase separation determines the final morphology of the phase separated mixture. A variety of morphologies have been found. The focus of the current work is on far off-critical mixtures in which the dextran concentration is much higher than the gelatine concentration. In such systems, phase separation below the gelation temperature of gelatine gives rise to a stable turbid fluid phase, which turns out to be a suspension of clusters of gelled gelatine spheres. The dependence of the sphere sizes and cluster sizes on cooling rate, gelatine/dextran molar ratio and total polymer concentration has been investigated and is tentatively explained in terms of the effect of the crosslinks of gelatine on the phase separation process.

#### A STUDY OF THE INTERACTION BETWEEN CHITOSAN AND LYSOZYME

HELMUT CÖLFEN<sup>a</sup>, STEPHEN E. HARDING<sup>a</sup>, DONALD J. WINZOR<sup>b</sup> and KJELL VÅRUM<sup>a</sup>

<sup>a</sup>National Centre for Macromolecular Hydrodynamics, University of Nottingham, Sutton Bonington LE12 5RD, UK

<sup>b</sup>Department of Biochemistry, University of Queensland, Brisbane QLD 4072, Australia

<sup>c</sup>Norwegian Biopolymer Laboratory (NOBIPOL), Division of Biotechnology, Norwegian Institute of Technology, University of Trondheim, 7034 Trondheim-NTH, Norway

Enzymic degradation of chitin by lysozyme reflects the hydrolytic specificity of this enzyme for  $\beta$ -1,4 linkages between the *N*-acetylglucosamine units that comprise the polysaccharide. The interaction of *N*-acetylglucosamine residues with

subsites C and E of lysozyme is required for catalysis and it is known that the strength of complex formation can be decreased by replacing the *N*-acetyl groups by protonated amino groups as a result of electrostatic repulsion between the polysaccharide polycation and the lysozyme at pH 4.5. Therefore the strength of the interaction between lysozyme and extensively deacetylated chitin (chitosan) is of interest. In this study analytical ultracentrifugation has been applied to study the extent of complex formation between both biopolymers in an acetate-chloride buffer, pH 4.5,  $I = 0.17$  (Cölfen *et al.*, 1994). Sedimentation velocity experiments using Schlieren- and UV-absorption optics with mixtures of 1 mg/ml chitosan and 0.1–0.6 mg/ml lysozyme give clear evidence for a chitosan–lysozyme interaction. Sedimentation equilibrium experiments on mixtures of 1 mg/ml chitosan and 0.3–0.6 mg/ml lysozyme employing the Rayleigh interference- and UV-absorption optics were analyzed by means of the 'Omega function' (Nichol *et al.*, 1976) to determine the fraction of free lysozyme in mixtures with defined total concentration. These analyses show that no free lysozyme is present in the mixtures independently of whether the whole mixture is monitored with the interference optics or only the lysozyme component with the UV-absorbance optics. A binding constant of at least  $10^5 \text{ M}^{-1}$  can be estimated. As the chitosan concentration in the molar scale is 45 times higher than the lysozyme concentration and the binding is nearly stoichiometric, the samples investigated represent a mixture of free chitosan and chitosan–lysozyme complexes. Quantitative description of this interaction would require the use of far smaller reactant concentrations than those detectable by current optical systems in the ultracentrifuge. Because the number of *N*-acetyl residues in chitosan is less than 1%, it becomes obvious that the acetylglucosamine residues are only a requirement for the catalysis, but not for the binding to lysozyme.

#### References

- Cölfen, H., Harding, S.E., Winzor, D.J. & Vårum, K. (1994). Evidence of strong interaction between lysozyme and extensively deacetylated chitin (submitted).  
Nichol, L.W., Jeffrey, P.D. & Milthorpe, B.K. (1976). *Biophys. Chem.*, **4**, 259.

#### CHARACTERIZATION OF GLIADIN–GALACTOMANNAN INCUBATION MIXTURES BY ANALYTICAL ULTRACENTRIFUGATION

A. SEIFERT<sup>a</sup>, L. HEINEVETTER<sup>a</sup>, H. CÖLFEN<sup>b</sup>, S.E. HARDING<sup>b</sup> and D.J. WINZOR<sup>c</sup>

<sup>a</sup>German Institute for Human Nutrition, Arthur-Scheunert-Allee 114-116, D-14558 Bergholz-Rehbrücke, Germany

<sup>b</sup>National Centre for Macromolecular Hydrodynamics, University of Nottingham, Sutton Bonington LE12 5RD, UK

<sup>c</sup>Department of Biochemistry, University of Queensland, Brisbane QLD 4072, Australia

The aim of this work is to examine the possible influence of the polysaccharide galactomannan (GAL) on the cereal protein gliadin (GLI) or a route to possibly helping patients with the coeliac disease known as gluten-induced enteropathy.

GLI and GAL in phosphate buffer (pH 6.5) and the incubated mixtures (1.67:1 wt/wt stirred for 3 h at 37°C) were investigated by analytical ultracentrifugation according to the

sedimentation velocity and equilibrium methods at 20°C. The plots of  $1/s_c$  vs  $c$  of both GAL and GLI-GAL mixtures after incubation show no significantly different shape suggesting the presence of no interaction products. According to the equation  $1/s_c = s_{20}^0 (1 + k_s c)$  values of  $s_{20}^0$  of  $(4.9 \pm 0.4)$  S,  $k_s = (664 \pm 47)$  ml/g and  $(4.7 \pm 0.2)$  S,  $k_s = (776 \pm 33)$  ml/g for GAL and GLI-GAL, respectively, were obtained. The concentration ranges of GAL ranged from 0.75 to 3.0 mg/ml for GAL alone and from 0.34 to 1.50 mg/ml in the incubated mixtures.

Low speed sedimentation equilibrium runs (Optima XL-A, Beckman) using the MSTARA computer evaluation program (Harding *et al.*, 1992) gave point (apparent) weight average molecular weights  $M_{w,app}$  of  $\approx 20,000$  g/mol for GLI and 200,000 g/mol for GAL. Investigating the GLI-GAL incubation mixtures, two non-interacting components were found with the same molecular weights as above.

#### Reference

Harding, S.E., Horton, J.C. & Morgan, P.J. (1992). In *Analytical Ultracentrifugation in Biochemistry and Polymer Science*, Harding, S.E., Rowe, A.J. & Horton, J.C. eds. Royal Society of Chemistry, Cambridge UK, Chapter 15.

#### THE EFFECTS OF SOLUTES ON THE GELATINIZATION OF SMOOTH PEA STARCHES

T.YA. BOGRACHEVA, S.P. LEONTIEV and YA.V. GENIN

*The Institute of Food Substances of RAS, 28 Vavilov Str., Moscow V 334 GSP I 117813 Russia*

When water is heated with a suspension of starch it undergoes a co-operative endothermic transition known as gelatinization. The molecular events responsible for this transition are uncertain, but entail melting of crystallites. Solute acts as a plasticiser in this process. Low concentrations of inorganic salts are known to raise the gelatinization temperature for both A- and B-types of starches, namely corn and potato starches. This study investigates the influence of inorganic neutral salts on the gelatinization process of C-type starches, isolated from smooth peas.

The starches of five peas varieties were studied. Wide-angle X-ray diffraction analysis shows that all of the starches have a similar degree of crystallinity (27–31%), however, they differ in the content of 'A' and 'B' polymorphs. So the content of 'B' polymorphs changes from 26 to 49%.

Quasi-equilibrium studies of the thermodynamic parameters of gelatinization of pea as well as potato and corn starches were made by DSC. On raising salt concentration both specific heat capacity increment ( $\Delta C_p$ ) and the specific enthalpy ( $\Delta H$ ) of gelatinization decreases for potato starch whereas this slightly increases for corn starch. At the same time both peak temperature ( $T_p$ ) and the difference between the termination and onset temperatures ( $\Delta T$ ) increases in a different manner for both starches. The addition of salt to C-type starches also results in the intake in  $T_p$ , at the same time the single peak becomes double.

A model was proposed, which represents the C-type starches as composed of two different types of independent cooperative structures, which contain either 'A' or 'B' polymorphs. The comparative analysis of  $T_p$ ,  $\Delta T$ ,  $\Delta C_p$ , and

$\Delta H$  for both pea starches with different contents of 'A' and 'B' polymorphs and potato as well as corn starches under different salt concentration was given. This analysis confirms the proposed model.

#### RHEOLOGY OF GUAR GALACTOMANNAN/RICE STARCH MIXTURES

PHILLIPPA RAYMENT, SIMON B. ROSS-MURPHY and PETER R. ELLIS

*Biopolymers Group, Division of Life Sciences, King's College London, Campden Hill Road, London W8 7AH, UK*

Biopolymer mixtures are of increasing importance in the design of model foods, for example, in the low fat spread area. Much of this work has been dedicated to mixed gel systems. However, the equally important area of dispersions of a soft filler phase in a biopolymer solution has been less well studied. For example, *in vitro* model systems are likely to be useful in studying the nutritional properties of water-soluble non-starch polysaccharides ('dietary fibre') in man. Foods containing guar gum are thought to reduce the postprandial rise in blood glucose and insulin levels (Ellis *et al.*, 1991) through the increase in viscosity of digesta in the stomach and small intestine (Roberts *et al.*, 1990). The presence of particulate material is likely to modify the rheological behaviour of guar gum and other such biopolymers dispersed in the aqueous phase of digesta.

In the present work, guar solutions, well recognised to be a model entanglement network system, were measured in both steady and oscillatory shear. A rice starch filler, selected for size and homogeneity of starch grain, was then added in small increments and the same experimental scheme applied. As the amount of filler is increased we would expect that the dispersion would begin to develop some additional features. Steady shear measurements of the pure guar galactomannan system show a Newtonian plateau at low shear rates followed by increasing shear rate dependence at high shear rates; the so-called 'power law' behaviour. This power law behaviour would be expected to become more pronounced at low shear rates as the starch filler concentration is increased. In oscillatory measurements, as the concentration of starch filler is increased,  $G'$  would be expected to increase faster than  $G''$  but with a reduction in the cross-over frequency. This suggests that the liquid-like behaviour at low frequencies changes to a more solid-like response at lower frequencies than the pure galactomannan system. In this poster we will present such data and discuss the interpretation in terms of, for example, a yield stress modified Cross equation.

**Acknowledgements** — The authors acknowledge the Ministry of Agriculture, Fisheries and Food and Leatherhead Food Research Association for financial support.

#### References

- Ellis, P.R., Dawoud, F.M. & Morris, E.R. (1991). *Br. J. Nutr.*, **66**, 363–379.
- Roberts, F.G., Smith, H.A., Low, A.G. & Ellis, P.R. (1990). In *Dietary Fibre: Chemical and Biological Aspects*, D.A.T. Southgate, K. Waldren, I.T. Johnson & G.R. Fenwick (eds). The Royal Society of Chemistry, Cambridge, pp. 164–168.