

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

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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

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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

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

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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