proteins, such as γ-globulin and BSA which have different isoelectric points.

INFLUENCE OF SUCROSE ON THE THERMODYNAMIC PROPERTIES OF THE 11S GLOBULIN VICIA FABA–DEXTRAN AQUEOUS SOLVENT SYSTEM

A.S. ANTIPOVA and M.G. SEMENOVA

Institute of Food Substances, Academy of Sciences of Russia, Vavilov Street 28, 117813 Moscow, Russia

Many processed and formulated foods are complex systems containing proteins, polysaccharides, lipids and low-molecular weight additives to provide taste and flavour. It is well known that the functional properties of biopolymers can dramatically change on altering the composition of the aqueous medium. The thermodynamic approach seems to be a fruitful approach to understanding the phenomena taking place.

One of the most widely used low-molecular weight food additives is sucrose. Sucrose content can reach very high levels in food. So for instance, sucrose content in ice-cream can be as high as 33% w/w and in different beverages concentrates up to 70% w/w.

In this connection we have attempted to study the influence of high levels of sucrose on the thermodynamic properties of the model system 11S globulin–dextran–water. It was established that addition of the sucrose to 50% w/v tends to significantly increase the solubility of the 11S globulin at pH 6·0 where protein has limited solubility in aqueous medium. In order to carry out thermodynamic investigations the borderline conditions for complete solubility of the 11S globulin in aqueous medium were chosen, namely, pH 7·0, I = 0·1 M. The thermodynamic parameters of the different types of pair interactions (the second virial coefficients) were estimated. The limit of thermodynamic stability of the systems (spinodal curve) and the coordinates of critical point were calculated. Experimental data were in good agreement with calculated results. It was observed that second virial coefficients of the 11S globulin and dextran greatly increased when the sucrose concentration in the aqueous medium was reduced below 50%. The cross second virial coefficient decrease indicated an increase in thermodynamic compatibility of the biopolymers in this case. Possible reasons for the phenomena observed are discussed.

Acknowledgment — The research described in this paper was made possible in part by Grant No. MLNOOO from the International Science Foundation.

MIXED GELS MADE FROM PROTEIN AND κ-CARRAGEENAN

R. IPSEN

Department of Food and Dairy Science, The Royal Veterinary- and Agricultural University, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark

A number of proteins are used in food products in order to provide increased functionality (waterbinding, gelation, emulsion stability etc.). Such functional proteins are often part of food systems, where hydrocolloids are also used, and a synergistic effect can be obtained (Marrs, 1989; Tolstoguzov, 1991).

Mixed gels with a total solids content of 18%, were made from soy or pea protein concentrates and κ-carrageenan and investigated using uniaxial compression and dynamic oscillatory measurements. Pea protein concentrate (PPC) exhibited greater synergy with κ-carrageenan than soy protein concentrate (SPC) in relation to gel strength, gel stiffness and pH stability. Application of modified Takanayagi models (Clark *et al.*, 1983) to oscillatory data indicated a shift in the continuous phase from protein to κ-carrageenan at concentrations from 4 to 8% κ-carrageenan in the total solids. This shift occurs at lower concentrations when PPC is used compared to SPC.

References

Clark, A.H., Richardson, R.K., Ross-Murphy, S.B. & Stubbs, J.M. (1983). *Macromolecules*, **16**, 1367–1374.

Marrs, W.M. (1989). *The British Food Manufacturing Industries Research Association, Scientific & Technical Surveys*, **168**.

Tolstoguzov, V.B. (1991). *Food Hydrocolloids*, **4**(6), 429–468.

STUDY OF THE COMPATIBILITY/INCOMPATIBILITY OF GELATIN/IOTA-CARRAGEENAN/WATER MIXTURES

C. MICHON, G. CUVELIER and B. LAUNAY

ENSIA, 1 avenue des Olympiades, 91305 Massy Cedex, France

A. PARKER[a] and G. TAKERKART[b]

[a]*R&D, Food Ingredients Division, Sanofi Bio-Industries, Baupte, Carentan, France*

[b]*Gelatin and Specialities Division, Sanofi Bio-Industries, 66, avenue Marceau, Paris, France*

The incompatibility of acid gelatin/iota-carrageenan mixtures has been studied. Both these biopolymers undergo a conformational coil/helix transition under suitable conditions of temperature and salt. In the helix conformation, the chains can form thermoreversible gels. As the coil/helix transition temperatures of the two biopolymers are different, so are the temperature domains of the sol–gel transition. The aim of this work was to study the concentration at which mixtures are incompatible and the influence of pH, salt, temperature and polymer molecular weight on the phase diagram.

Three series of mixtures were studied:

(1) gelatin and iota-carrageenan in distilled water without pH adjustment;
(2) the same mixtures adjusted to pH 6·5;
(3) the same mixtures adjusted to pH 6·5 and 0·2 M in sodium.

We have used two different molecular weight samples of both gelatin (g2 and g3) and carrageenan (i1 and i2). Three types of mixtures were studied: g2/il; g3/il; g3/i2. The influence of temperature on the phase diagram was observed between 20 and 70°C. At each temperature, mixtures were either clear or showed incompatibility in several ways: cloudy one-phase systems or two separate phases with each phase either gelled or ungelled.

Incompatibility occurred over a large range of concentrations for mixtures prepared in distilled water. Compatibility

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[a], STEPHEN E. HARDING[a], DONALD J. WINZOR[b] and KJELL VÅRUM[a]

[a]*National Centre for Macromolecular Hydrodynamics, University of Nottingham, Sutton Bonington LE12 5RD, UK*
[b]*Department of Biochemistry, University of Queensland, Brisbane QLD 4072, Australia*
[c]*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 β-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 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[a], L. HEINEVETTER[a], H. CÖLFEN[b], S.E. HARDING[b] and D.J. WINZOR[c]

[a]*German Institute for Human Nutrition, Arthur-Scheunert-Allee 114-116, D-14558 Bergholz-Rehbrücke, Germany*
[b]*National Centre for Macromolecular Hydrodynamics, University of Nottingham, Sutton Bonington LE12 5RD, UK*
[c]*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