measure diffusion coefficients in gel systems using a confocal laser scanning microscope is described. Diffusion coefficients (D), were determined for fluorescently labelled dextrans of varying molecular weight in agarose gels. The results were compared with the 'repeated line scans' method developed from studies using epi-fluorescence microscopy by Henry *et al.* (1992) and the classical method based on the 'double diffusion cell' e.g. Bain *et al.* (1992).

Good agreement was achieved between the latter method and CFMM for all the dextrans studied. The repeated line scan method gave higher diffusion coefficients for the lower molecular weight dextrans possibly because of interfacial tension and swelling effects which aggravate the curved meniscus surface.

The CFMM technique was experimentally simple, involved only a single image and enabled diffusion coefficients to be determined rapidly at defined microscopic locations within gel systems.

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COMPOSITIONAL MAPPING OF MIXED GELS USING FTIR MICROSPECTROSCOPY

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We have developed a technique to product compositional maps of phase-separated protein/polysaccharide mixed gels using Fourier transform infrared (FTIR) microspectroscopy. The maps plot out the composition of either the protein, the polysaccharide or the water as a function of position in the sample. The maps can be presented in the form of two-dimensional contour plots or three-dimensional surface plots.

The technique is automated and uses an FTIR microscope interfaced to a motorised stage. The stage drives a sample in stepwise fashion to cover a two dimensional grid of points across the sample. At each position in the grid, the FTIR microscope records the infrared spectrum over a cross-sectional area of sample, the size of which is specified by apertures in the microscope.

We have used the technique to produce compositional maps of the amylopectin/gelatin and dextran/gelatin systems using 40 μm × 40 μm apertures with a grid step size of 40 μm. Compositional maps were generated in the first instance by simply plotting the area of an infrared absorption peak from one of the components in the sample. Fully quantitative compositional maps in terms of actual concentration were also produced by analysing the spectra with the method of partial least-squares (PLS).

We recently showed how PLS analysis can be used in conjunction with FTIR spectroscopy to plot the phase diagram of bulk phase-separated solutions above the gel temperature of both components. Our mapping technique therefore allows the compositions in a gel to be directly compared with those reached at equilibrium in the absence of gelation, using the same molecular probe, namely, infrared radiation. Furthermore, our technique can be applied to any other protein/polysaccharide mixture – provided that phase separation takes place on a scale larger than the diffraction limit of infrared radiation (~20 μm).

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PHASE SEPARATION IN AQUEOUS PROTEIN–POLYSACCHARIDE SYSTEMS

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Solutions containing two different polymers frequently exhibit incompatibility resulting in the formation of two liquid layers, with each layer enriched in one or other of the polymers. We have observed such behaviour upon mixing aqueous solutions of globular proteins such as bovine serum albumin (BSA) with neutral polysaccharides notably dextran and hydroxyethyl cellulose (HEC).

Several factors have been found to affect the phase separation of such protein–polysaccharide systems; pH, polysaccharide structure and molecular mass and addition of salt.

Phase separation occurs most readily at the pH corresponding to the protein isoelectric point. As the pH moves away from the isoelectric point phase separation is suppressed and a one phase system results. Phase diagrams have been constructed which show that HEC produces phase separation at lower concentrations than dextran of similar molecular mass. An explanation may derive from the fact that HEC is a fairly rigid linear polymer in solution, whereas dextran is a more flexible and compact molecule. The effect of polysaccharide molecular mass on the phase behaviour is also more pronounced for the systems containing HEC than dextran. The phase diagrams of the BSA – dextran 250 and BSA – dextran 2000 systems are almost identical, whereas phase separation was found to occur at progressively lower HEC concentrations as the molecular weight increased from 64,000 to 450,000.

Addition of salt to a phase separated system at the protein isoelectric point has been found to result in a one-phase system. This is reported to be as a result of shielding of the dipolar attractions between protein molecules. At a pH above or below the isoelectric point the protein molecules are charged and electrostatic repulsive forces exist preventing phase separation. Salt ions would be expected to shield these forces resulting in a two-phase system. However, this was not observed in the BSA – dextran mixture at pH 6·0. This may be due to binding of the anions to the protein which in effect shifts the isoelectric point to a more acidic pH.

The dependence of phase separation on a number of factors opens up the possibility of selective protein concentration or isolation by addition of polysaccharide to mixtures of

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

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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.

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MIXED GELS MADE FROM PROTEIN AND κ-CARRAGEENAN

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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.

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

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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