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.

References

Bain, J.C., Ganderton, D. & Solomon, M.C. (1990). *J. Biopharm. Sci.*, **1**, 225–234.

Henry, B.T., Adler, J., Hibberd, S., Cheema, M.S., Davis & Rodgers, T.G. (1992). *J. Pharm. Pharmacol.*, **44**, 543–549.

COMPOSITIONAL MAPPING OF MIXED GELS USING FTIR MICROSPECTROSCOPY

C.M. DURRANI and A.M. DONALD

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

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).

References

Durrani, C.M. & Donald, A.M. (1994). *Macromolecules*, **27**, 110–119.

Durrani, C.M., Donald, A.M., Prystupa, D.A. & Clark, A.H. (1993). *Macromolecules*, **26**, 981–987.

Infrared Microspectroscopy: Theory and Applications (1988). Messerschmidt, R.G. & Harthcock, M.A., eds. Marcel Dekker, New York.

PHASE SEPARATION IN AQUEOUS PROTEIN–POLYSACCHARIDE SYSTEMS

A.R. HOSKINS, I.D. ROBB and P.A. WILLIAMS

Centre for Water Soluble Polymers, The North East Wales Institute, Connah's Quay, Deeside, Clwyd CH5 4BR, UK

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