

DOCTORAL THESIS DEFENSE OF GIREESHKUMAR BALAKRISHNAN NAIR

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Speciality : CHIMIE ET PHYSICO-CHIME DES POLYMERES

Particle diffusion in protein gels and at interfaces

submitted to the examining committee

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Abstract : Knowledge of the mobility of particles in materials is of prime importance in many applications. This research presented in this thesis aimed at exploring the possibilities of using confocal laser scanning microscopy (CLSM) to measure the displacement of tracer particles in two systems : gels formed by heating globular proteins in solution and water in water emulsions formed by incompatible polymer solutions. With first system displacement of tracers in three dimensions through a matrix was studied, while with the second system two dimensional displacement of particles at the water-water interface was probed.

We used β -lactoglobulin gels as a model system to investigate the diffusion in gels because its structure can be fine tuned by varying salt or pH. In addition, its structural properties at various conditions have already been investigated in detail. Globular protein gels with a variety of structures were prepared by heating β -lactoglobulin solutions at different concentrations ($C=20-120$ g/l) and different ionic strengths ($C_s=0-0.25$ M) at pH 7. It was found that the visual aspect of the gel varied from transparent to turbid with increasing salt concentration. Transparent gels are homogeneous ($C_s \leq 0.15$ M) whereas turbid gels ($C_s > 0.15$ M) are heterogeneous. The structure of the heterogeneous gels was analyzed in terms of the pair correlation function of the protein concentration, and the volume fraction of the gels was determined. For homogeneous gels scattering measurements were used to characterize the structure.

The diffusion of particles was investigated both in homogeneous and heterogeneous gels by combining CLSM with multiple particle tracking (MPT) and fluorescence recovery after photobleaching (FRAP). With MPT, the mean square displacement (MSD) of fluorescent polystyrene latex particles with radii 0.1 to 1 μm was determined in solutions and gels from the CLSM images using a particle tracking routine. With FRAP, the intensity recovery of a region of interest after bleaching was monitored in solutions and gels for fluorescein labeled dextran molecules with different radii (5 to 50nm). It was found that MPT is not a useful tool to study the diffusion of tracers in homogeneous protein gels since the particles are trapped in the gel matrix, but FRAP was a useful method because smaller tracers could be used.

It was found that at higher salt concentrations ($C_s > 0.15$ M) the gel structure was not strongly correlated with the protein concentrations since the diffusion coefficient of tracer in gels normalized by their diffusion coefficient in water (D/D_0) was almost independent of C , but at lower salt concentrations there was a strong decrease of D/D_0 with increasing C . A remarkable observation was that the diameter of the tracers that were trapped was only weakly correlated to the correlation length and hence the average pore size of the gel. Probably distribution of pore sizes is an important factor. FRAP measurements showed that the fraction of mobile tracers decreased first with increasing salt concentration up to $C_s=0.02$ M, but increased at $C_s > 0.05$ M. The minimum can be attributed to a combination of the increase of both the average correlation length and the width of pore size distribution which shows again that it is important to consider both the average pore size and its distribution.

The water in water emulsions formed by mixing aqueous solutions of PEO and dextran, to which fluorescent polystyrene latex particles were added with radii 0.1 to 1 μm . The particle and dextran phase were visualized separately using multi fluorescent labeling techniques. Particles with radii as small as 0.1 μm could be trapped at the interface between PEO and dextran rich phases with interfacial tensions as low as 10^{-6} N/m. Trapping of the particles at the interface in water in water emulsions could be explained by the reduction interfacial tension in spite of the fact that it was orders of magnitude smaller than in oil-water emulsions. Various degrees of coverage could be observed up very dense with hexagonal crystalline order. At intermediate coverage transient clustering was observed, while at low coverage the diffusion coefficient of the particles at the interface could be determined using MPT. D was found to be determined by the viscosity of the bulk phases without a specific effect of the interface.