Abstract. We report static fluorescence quenching experiments in the ionic AOT and the nonionic Igepal water-in-oil microemulsions and show that compartmentation, which is well known in the AOT system, is also effective in Igepal/cyclohexane, but not in water-rich Igepal/n-hexane microemulsions. We investigate the reactions of metal-ion binding to murexide, using the pressure jump relaxation method. Strong interactions with the ionic surfactant decrease the ligand binding strength in the AOT microemulsion and droplet collision determines the kinetics when the reaction is fast. No interactions and no rate limiting steps are observed in the Igepal microemulsions. We further investigate the reactions of the solubilized ferriheme proteins methemoglobin and metmyoglobin with the ionic ligands azide and fluoride. In Igepal microemulsions the proteins behave similarly to the aqueous solution. In the AOT system they do not have their native form and show optical absorption spectra dependent on the size of the water pool. This may be interpreted as an AOT induced shift of the monomer-dimer equilibrium of hemin adsorbed at the denatured protein.

Introduction. Many investigations have been undertaken to confine proteins in artificial compartments as lipid vesicles and water-in-oil microemulsions with the aim to study the influence of the environment on the structure and reactivity of the protein (for a review see ref 1). We report here (2) on the solubilization and the properties of Methemoglobin and Metmyoglobin in microemulsions of the type water/Aerosol-OT/n-heptane and water/IgepalCO520/n-hexane and c-hexane resp. The Ferriheme proteins contain iron in the oxidized form which complexes a water molecule or a variety of small negative ions. The heme absorption spectrum and the binding properties may be a sensitive indicator of structure changes as internal hemichrome formation and denaturation.
We perform a relaxation kinetic investigation using the pressure jump technique with optical detection. The method is suitable for nonconducting microemulsions; also for electrical conducting microemulsions it may be more adequate than temperature jump experiments, since light scattering relaxation modes—which in the Igepal/n-hexane system are in the millisecond range—are initiated by temperature rather than by pressure changes. In order to test our experimental setup, which is especially designed for nonaqueous solutions, and obtain information on the structure and dynamics of the microemulsions we have performed measurements of metal-ion binding to murexide; this system has been characterized before in AOT microemulsions with other kinetic techniques (3).

Compartmentation is quite well characterized in AOT microemulsions; water is solubilized as small droplets whose radii \( r \) depend on the molar water to surfactant ratio \( R \) according to \( r = 0.18*R \) (3,4). With Igepal microemulsions the compartmentation is less defined; freeze fracture micrographs (5) give an idea of the water and oil domains in various regions of composition. We report fluorescence quenching experiments in Igepal/c-hexane which give evidence to a certain compartmentation within the lifetime of the fluorescent probe.

Materials and methods. Human Hemoglobin has been prepared from blood by standard procedures, oxidized by ferricyanide and freed from oxidation products and small molecules by means of a sephadex G25 column.

Freeze dried sperm whale Metmyoglobin was obtained from Serva; the surfactants AOT (Aerosol-OT, 1,4-bis(2-ethylhexyl)sulfosuccinate/Sigma), IgepalCO520 (Pentaethyleneglycol-4-(n-nonyl)phenylether/Aldrich) and DDAB (Di-dodecyl(dimethylammonium-bromide/Kodak) were used without further treatment. Microemulsions were prepared by mixing the aqueous solution with the surfactant/oil solution. In 0.3M AOT/n-heptane and 0.3M Igepal/c-hexane \( R \) values from zero to about 60 and 30 resp. can be reached at 20°C, before phase separation occurs. The 15wt\% Igepal/n-hexane solution can solubilize water in the range from \( R= 65 \) to 95 at 25°C (cf ref 6). The fluorophore \([\text{Ru(bipy)}_2]\text{Cl}_2\) was obtained from Strem and purified by recrystallization from water. Solutions were kept in the dark in order to avoid light induced degradation effects.

Fluorescence spectra and quenching experiments were performed with an Aminco-Bowman fluorimeter equipped with the ratio unit. Nitrogen, saturated with the organic solvent, was passed through the solution for several minutes until a constant fluorescence signal was obtained.