



## Ultra structure of oil-in-water emulsions - a comparison of different microscopy- and preparation methods

Jensen, Louise Helene Søgaard; Loussert, C.; Horn, Anna Frisenfeldt; Jacobsen, Charlotte; Nielsen, Nina Skall; Humbel, B.M.; Horsewell, Andy

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# Ultra structure of oil-in-water emulsions

## a comparison of different microscopy- and preparation methods

L.H.S. Jensen<sup>1</sup>, C. Loussert<sup>2</sup>, A.F. Horn<sup>3</sup>, C. Jacobsen<sup>3</sup>, N.S. Nielsen<sup>3</sup>, B.M. Humbel<sup>2</sup> and A. Horsewell<sup>4</sup>

<sup>1</sup> Center for Electron Nanoscopy, Technical University of Denmark, <sup>2</sup> Electron Microscopy Facility, Université de Lausanne, Switzerland, <sup>3</sup> National Food Institute, Technical University of Denmark, <sup>4</sup> Department of Mechanical Engineering, Technical University of Denmark

### Introduction

Addition of fish oil to industrially prepared food products is attractive to the food industry because of the well-documented health effects of long chain polyunsaturated omega-3 fatty acids in the fish oil. However, omega-3 fatty acids are highly susceptible to lipid oxidation because of their many double bonds, and the incorporation of fish oil into food products is therefore limited by the development of unpleasant off-flavours. It has been shown that addition of fish oil to selected food products as emulsions rather than crude oil changes the products' stability against oxidation but whether emulsification is an advantage or not seems to depend on the food matrix to which the emulsion is added<sup>(1,2)</sup>. Hence, we sought to determine the factors which controls the oxidation in pure emulsions. A schematic overview of an emulsion is shown in figure 1. It has been proposed that oxidation of oil-in-water emulsions is initiated at the interface between oil and water and that the oxidation is to some extent dependent on the micro structure of the emulsion; including the size of oil droplets, the emulsifier type and the thickness of the interface. Also the distribution of the emulsifier in the water phase might affect the oxidation since some emulsifiers have antioxidant properties, e.g. sodium caseinate is a metal chelator which can sequester transition metals that would otherwise act as prooxidants. The potential link between the oxidative stability and the interface structure is not well understood. The main objective of this study is to characterize the structure of fish oil emulsions with respect to oil droplet size, distribution, and ultimately to view the structure and thickness of the interface layer as well as the emulsifier in the water phase. We present here a model emulsion with 1% sodium caseinate and 10% oil. The mean droplet diameter measured by laser diffraction is 135 nm and the texture is like milk. The size of the structures of interest and the texture poses some challenges for the sample preparation, e.g. the need for a matrix to hold the sample during chemical fixation and we have compared different methods for preparation of the structure in order to find a best practice.

### Casein

The protein-based emulsifier sodium caseinate is derived from casein by removal of calcium phosphate. In the absence of calcium phosphate, casein is flexible due to the absence of notable 2' structure and is able to stretch and change shape at the interface according to the concentration<sup>(3)</sup>. The native casein micelle is shown in figure 2. Figure 3 shows the casein in the water phase of a sample that has been prepared by mixing. In contrast, the emulsion presented below was prepared by high pressure (69 MPa) to create the small droplets.

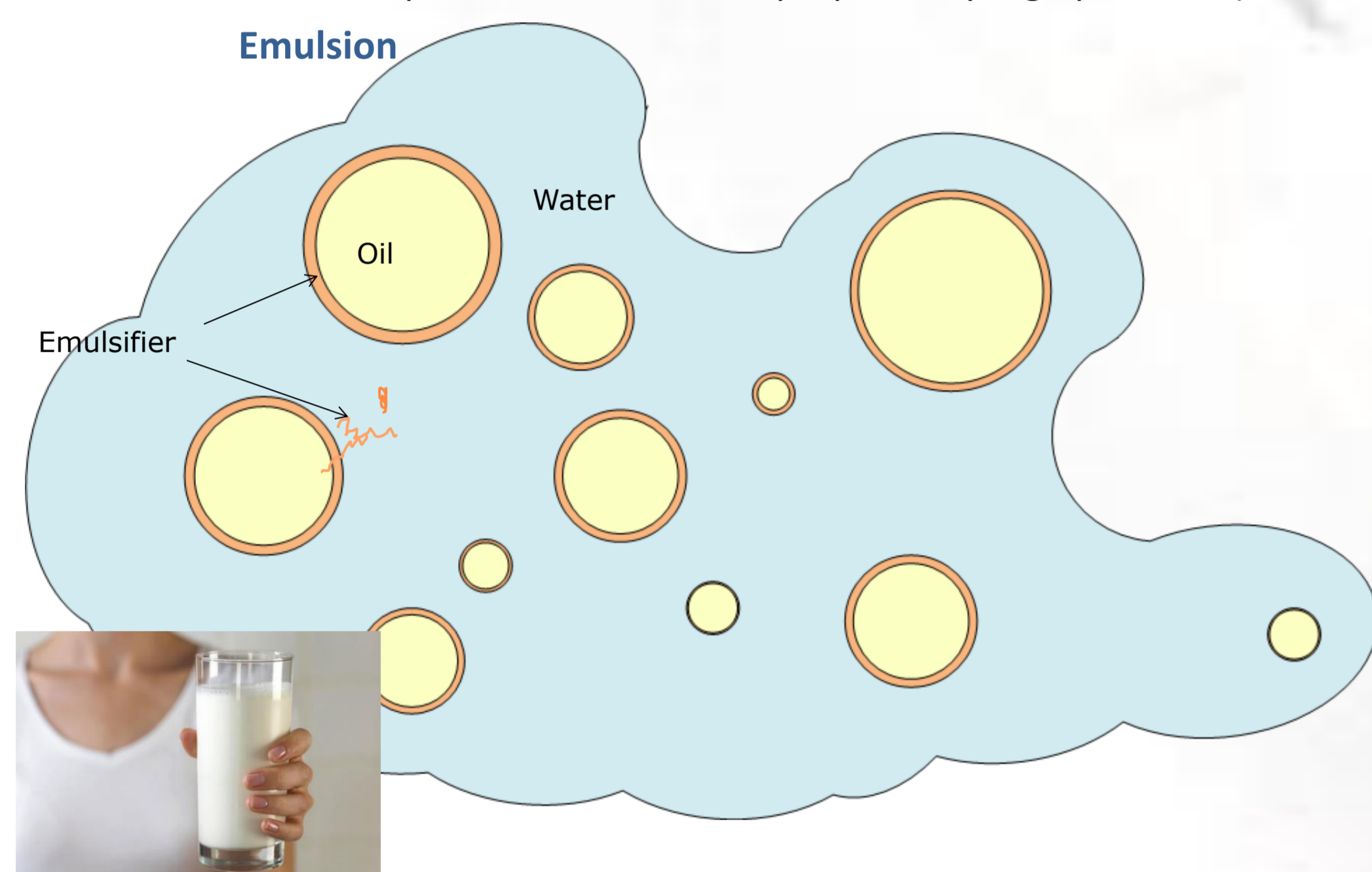


Figure 1. Schematic oil-in-water emulsion. The emulsions presented below is stabilized by sodium caseinate. The texture of the emulsion is like that of milk, it contains 10% fish oil in droplets of a mean diameter of 135 nm.

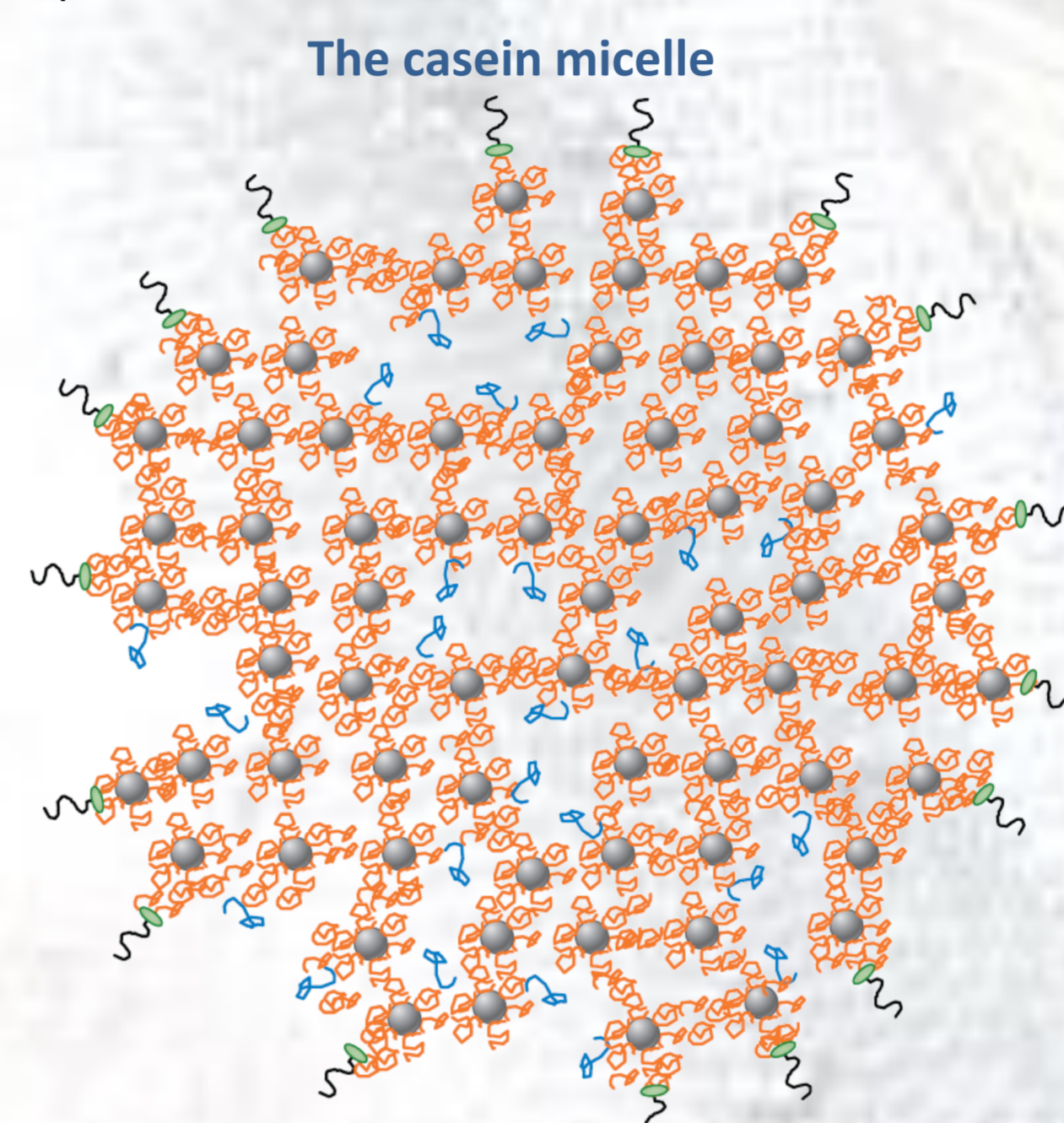


Figure 2. The native casein micelle<sup>(4)</sup> Grey particles are calcium phosphate.

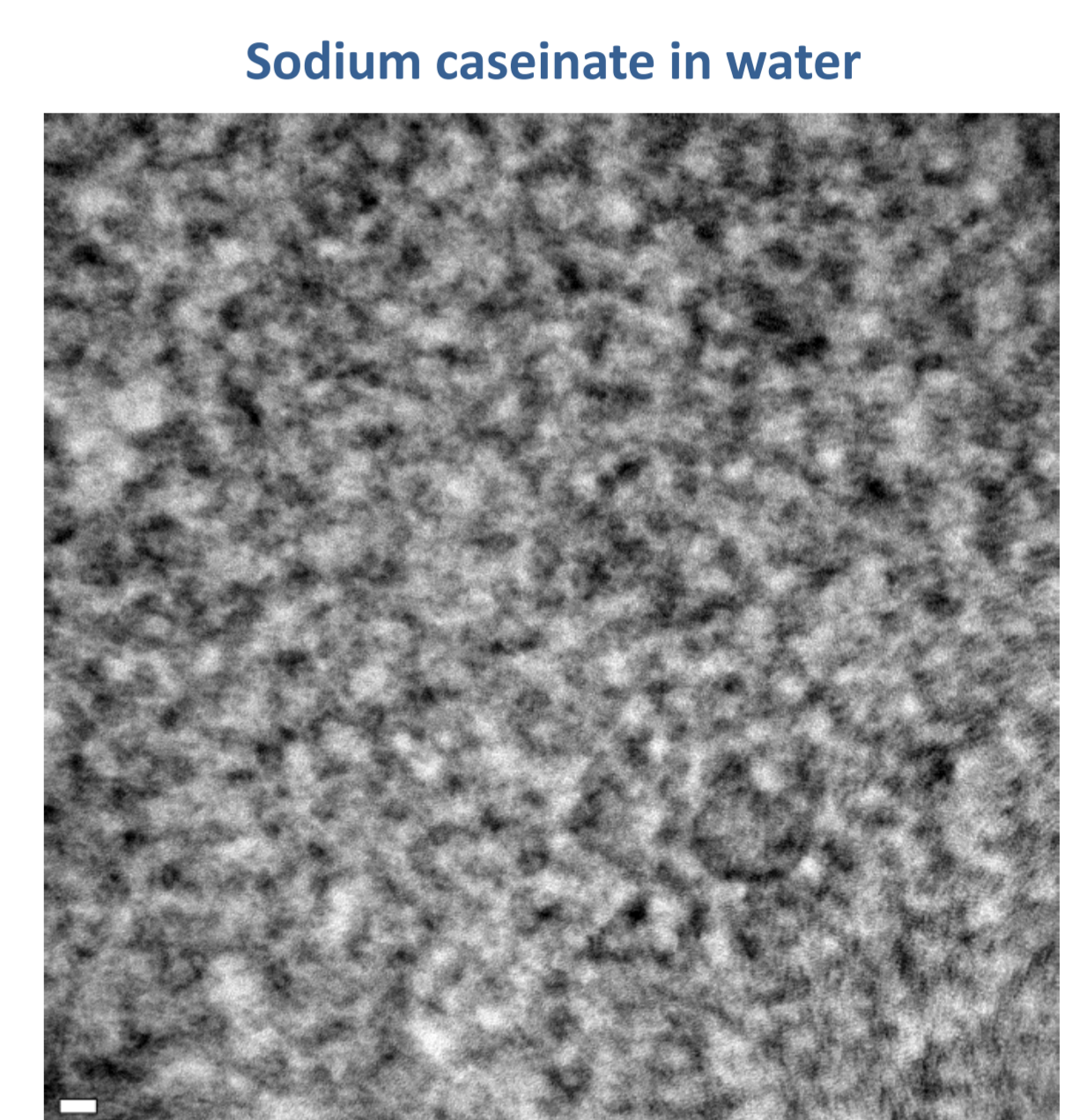


Figure 3. Sodium caseinate in the water phase. Scale bar 20 nm. High pressure frozen and freeze substituted sample.

### Chemical fixations in agar

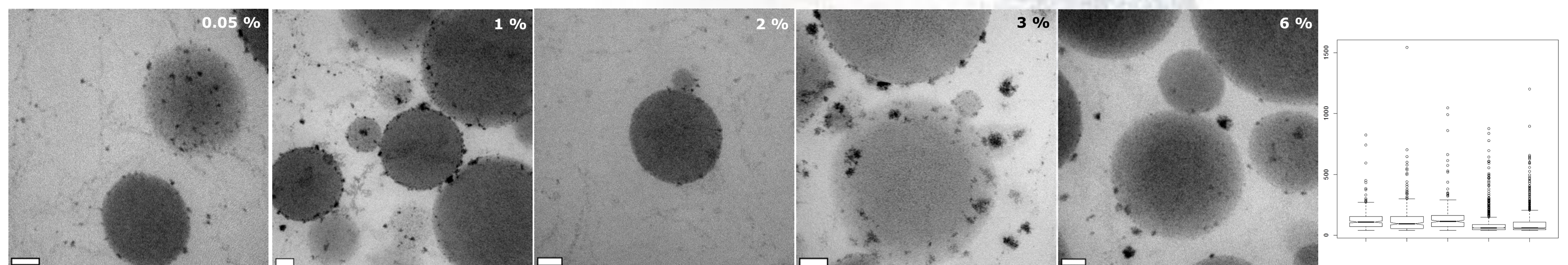


Figure 4. The chemically fixed samples were mixed with low-melting agar 1:1 and cut into tiny cubes prior to chemical fixation. The agar-mixed samples were used to determine a suitable concentration of glutaraldehyde in combination with 2.5% paraformaldehyde. The droplets were shrunk as an effect of the concentration of glutaraldehyde. 2 groups were found, i.e. [0.05%, 1%, 2%] and [3%, 6%] which did not differ within but the groups were significantly different by a confidence interval of 0.95. The first group had diameters in the range of 96-116 nm whereas the second group had diameters of 60-61 nm. A concentration of 3% was selected also based on previous fixation experiments of emulsions with larger droplet sizes that showed insufficient fixation below 3% glutaraldehyde (data not shown). In all cases, condensed protein aggregates were seen to be collapsed on the droplet surfaces.

### Chemical fixations in agar pockets (A&B)

### Chemical fixations in capillary tubes (C&D)

### High pressure freezing and freeze substitution

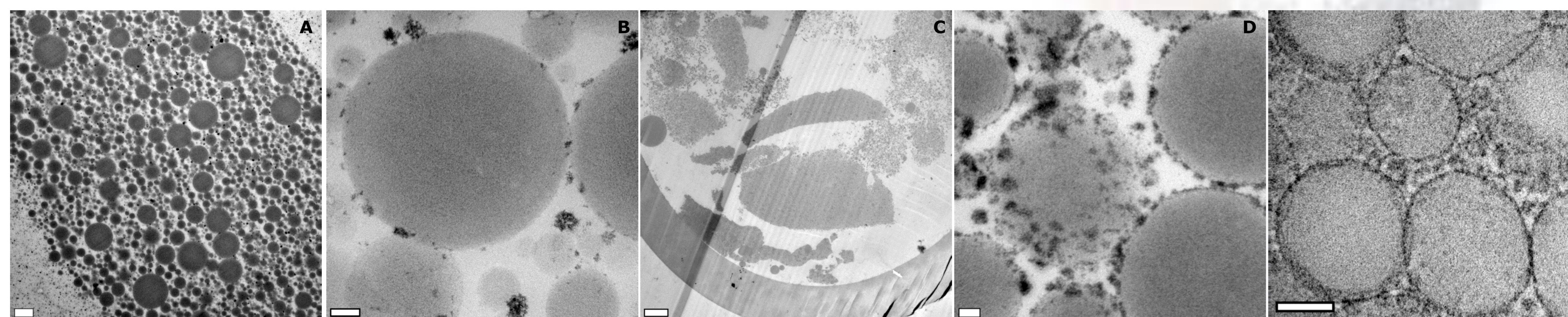


Figure 5. Two matrices were tested to hold the sample during chemical fixation by the previous determined glutaraldehyde concentration; either agar pockets (A & B) or capillary dialysis tubes (C & D). Agar pockets were casted in silicone rubber wells between two microscope glass slides and holes were punched by a biopsy needle. These small wells were filled with sample and sealed in both ends by agar, after which the small pockets were cut from the agar slab and fixed chemically. This method showed good preservation of the sample and in this case protein aggregates were also seen on the droplet surfaces. The samples inside the capillary dialysis tubes were aggregated inside the tubes (cross section of tube seen in C) but the samples were not distorted. Furthermore, the capillary tubes retained a larger amount of protein aggregates (D).

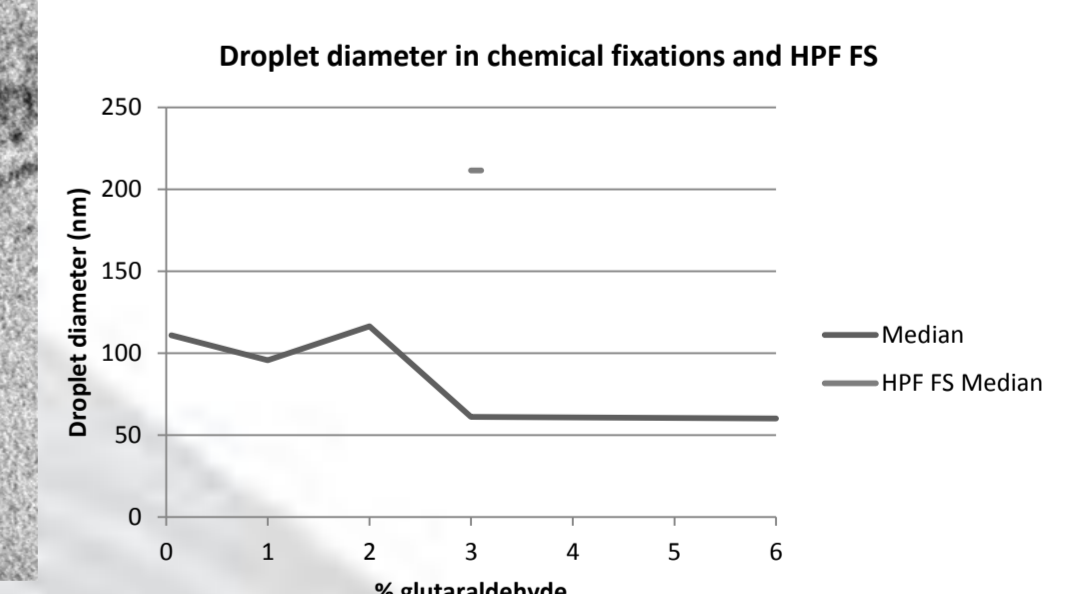


Figure 6. The sample was high pressure frozen and freeze-substituted. Freeze substitution did not cause the droplets to shrink so much as the chemically fixed samples. Furthermore the protein aggregates were not shrunk and condensed as in the chemically fixed samples but made a sub-population of mini micelles which correspond to the reaction of the native casein to high pressure. By the contraction of the micelles the mean diameter was 210 nm.

### Conclusions

For the best preservation of the entire sample, oil droplets as well as the continuous water phase, the preferred method would be high pressure freezing and freeze substitution since this method did not shrink the droplets to a degree as the chemically fixed samples. Furthermore, the method showed good preservation of the protein in the water phase which apparently had formed mini micelles due to the application of high pressure. This might have implications for the antioxidative effect of the emulsifier. However, for visualization of the oil droplets, chemical fixation and room temperature embedding should be sufficient, as long as the shrinkage is taken into account.

### References

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