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A THIN LAYER SAMPLE GUIDE FOR THE INVESTIGATION OF FLUIDS BY ANOMALOUS X-RAY SCATTERING

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Anomalous X-ray scattering allows the separation of the scattering of special elements from the scattering of complex materia, e.g. biological macromolecules (proteins, nucleic acids or membranes) or polymers [1]. Many proteins (enzymes) contain a few number of those atoms, which are not present in the over all structure, within specialized regions of functional relevance, the catalytic centers. Thus the localization of those atoms by anomalous X-ray scattering yields a functional relevant part of the molecular structure, which is in most cases not accessible by other methods. Sensitive biological complexes, e.g. membranes, and many proteins cannot be crystalized in their native state. This problem can be overcome by investigation of these macromolecules by X-ray small angle scattering of aqueous solutions. Many biologically relevant elements, e.g. calcium, sulphur and phosphorus, show anomalous X-ray scattering at long wavelength (0.3-0.6 nm). Unfortunately the absorption of low energy X-ray radiation by matter is so high, that only thin layers of material can be investigated at long wavelength. Thus we have constructed a new type of sample guide sufficient for the investigation of thin layers of fluids.

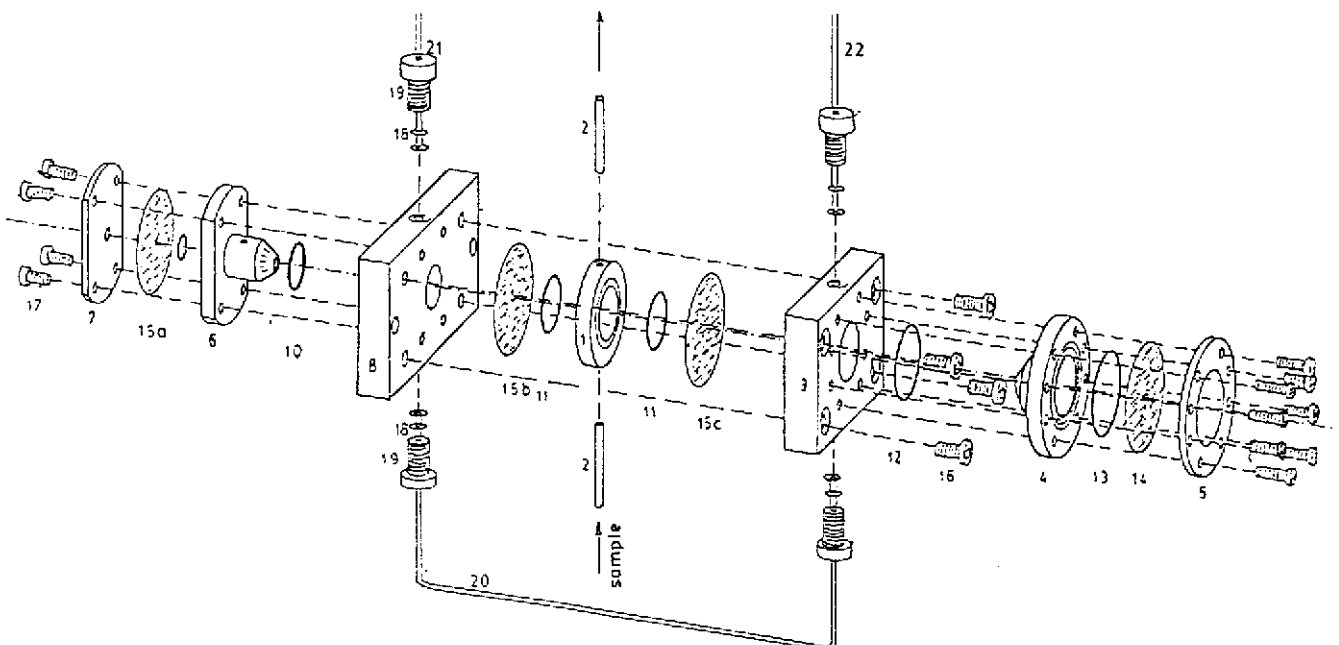


Fig. 1: Expanded view of the thin layer sample guide. The beam passes four mylar windows (15a, 15b, 15c, 14) and the sample (within the ring (1)) from the left. The space between the windows is filled with helium.

As shown in Fig.1, the sample guide consists of an aluminium case (3,8), which carries a fixed and an adjustable beam guide (4,6). The beam, coming from the left, passes four mylar windows (15a, 15b, 15c, 14). The space between the window pairs (15a, 15b and 15c, 14) is filled with helium (1 bar), which is supplied by teflon tubes (20-22). The fluid sample is supplied by other teflon tubes (2) and located within the sample ring (1). In the middle of the ring, the windows (15b, 15c) and the sample are compressed to a thin layer (25-1000 μm) by the beam guides. The thickness of the layer is adjusted by four screws (17).

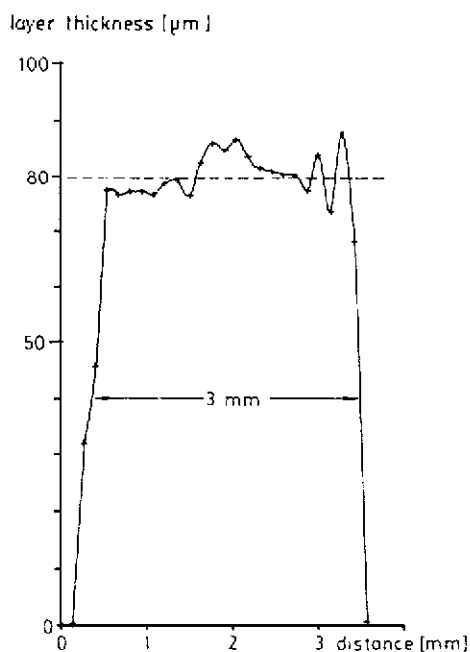


Fig. 2: The light absorption of a dye solution indicates that the thickness of the fluid layer is nearly homogenous.

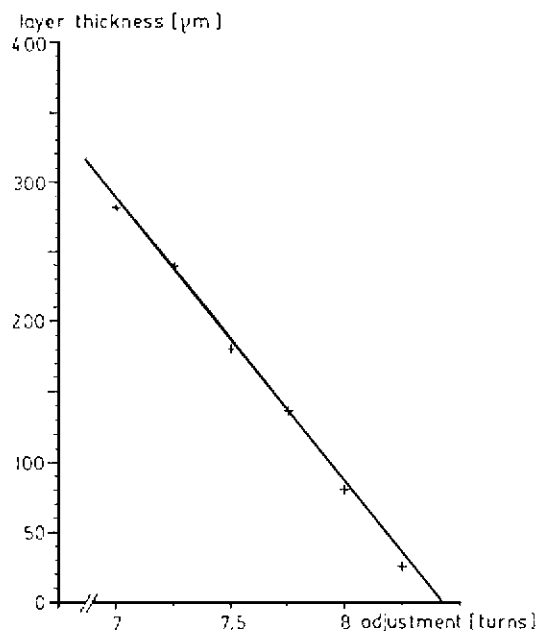


Fig. 3: The thickness of the fluid layer is adjustable between 1 mm and 25 μm .

The distribution of the thickness of the fluid layer within the holes in the beam guides (3 mm) is measured by scanning the optical density of the sample guide filled with a dye solution in a special adjustment run. In Fig.2 the homogeneity ($\pm 5 \mu\text{m}$), which in this case was adjusted to 80 μm thickness, is shown. Possibly the small fluctuations in the light absorption are due to reflections on the mylar windows and only partially to thickness fluctuations. As shown in Fig.3, the thickness of the fluid layer can be adjusted down to 25 μm . Such thin layers are sufficient for scattering experiments at large wavelength (0.5-0.6 nm), e.g. anomalous scattering of sulphur and phosphorus. Some samples can be investigated in the presence of the dye. In case of proteins, sensitive to the dye, the sample guide is carefully cleaned and filled with the sample solution without variation of the layer thickness.

In first experiments, the anomalous X-ray scattering of nucleotides (CaATP), model systems of biological membranes (lipid vesicles) and calcium binding proteins (F_1 ATPase and Calmodulin) have been investigated.

1) Stuhmann, H.B. (1985) *Advances in Polymer Sciences* 67, 124