19.3.3.3. *Experimental considerations*

19.3.3.3.1. *Sample preparation*

Sample volumes required for one measurement are between 10 to 50 µl, occasionally more, depending on the specific design of the sample cell used. The concentration required to record a scattering curve with satisfactory statistics depends primarily on the molecular weight of the protein and the beam flux. Approximately 1 mg ml^{-1} of a small protein (10–20 kDa) is the lower limit for recording a scattering pattern with satisfactory statistics when experiments are performed with a typical synchrotron instrument equipped with a gas-chamber detector. Somewhat lower concentrations of larger molecular weight proteins may be used. Higher concentrations will improve statistics significantly and reduce exposure times, but interparticle interference may result from high concentrations. Time-resolved experiments benefit dramatically from higher sample concentrations. In addition to the scattering power of the sample, the signal-to-background ratio and overall stability of an instrument (from X-ray source and optics to detector) limit the lowest concentration for a given experiment. Although higher concentrations add dramatically to the scattering and improve statistics, sample solutions must be monodisperse. Small-angle solution scattering is not well suited to the study of polydisperse systems, which give scattering of the entire molecular population weighted by the square of the mass, although a few distinct populations of substantially different sizes may be resolved with good-quality data. Chemical components that may have been carried along in a sample preparation, such as ammonium sulfate, sucrose, chloroform or caesium chloride, should be removed. The presence of such compounds may change the electron-density contrast and X-ray absorbency of the sample. In general, this can be most effectively avoided by exhaustive dialysis with the desired buffer solutions. The outer solution used for the final dialysis should be used for the blank measurements. Scattering contributions from the buffer solution, the sample cell and parasitic scattering must be subtracted from the measured scattering curve; these can be measured accurately from a well prepared blank. Extra buffer solution should be available for sample dilution. The data quality is improved and problems with radiation-sensitive samples are readily detected when protein concentrations and biological activities of samples are measured before and after the scattering experiment. Accurate protein concentration measurements permit scattering intensities from different samples to be scaled together accurately. This is particularly important in determining molecular weight.

19.3.3.3.2. *Sample-handling devices*

Sample holders used in solution scattering are either Lindeman glass or quartz capillaries, or machined cells equipped with flat windows (Fig. 19.3.3.6). Glass capillaries have been widely used to contain a sample solution. A beam size significantly smaller than the diameter of the capillary is required to minimize strong parasitic scattering from the round edge of the capillary. A large beam size in the horizontal direction may be used with capillaries to obtain stronger scattering intensity without adding parasitic background. Capillary cells are suitable for measuring scattering primarily in the direction perpendicular to the long axis of the cell. The advantage of the capillary cell is the small volume of sample solution required for measurements. About 4 µl of a sample solution in a 1 mm-diameter capillary provides sufficient material for the experiment. By employing a specialized holder, a capillary cell can be placed under a vacuum, minimizing air scattering (Dubuisson *et al.*, 1997). This feature is useful for solution scattering studies of small proteins. Anaerobic samples may be sealed in a capillary.

Another common sample cell holds the solution between a pair of flat windows. This cell offers two improvements over capillary cells – a larger beam cross section may be used, which increases the number of photons incident on the sample, and a two-dimensional detector can be effectively used for recording the scattering. Flat-window cells are available that require only about 10 µl of solution. Both capillary and flat-window cells require a holder with temperature regulation. The choice of window materials for the sample container is important because of the weak sample scattering. An X-ray-transparent material is required that has little intrinsic scattering within the scattering-angle range of the sample. Common window materials include synthetic mica of high purity and certain types of polypropylene and polyamide. Etched high-

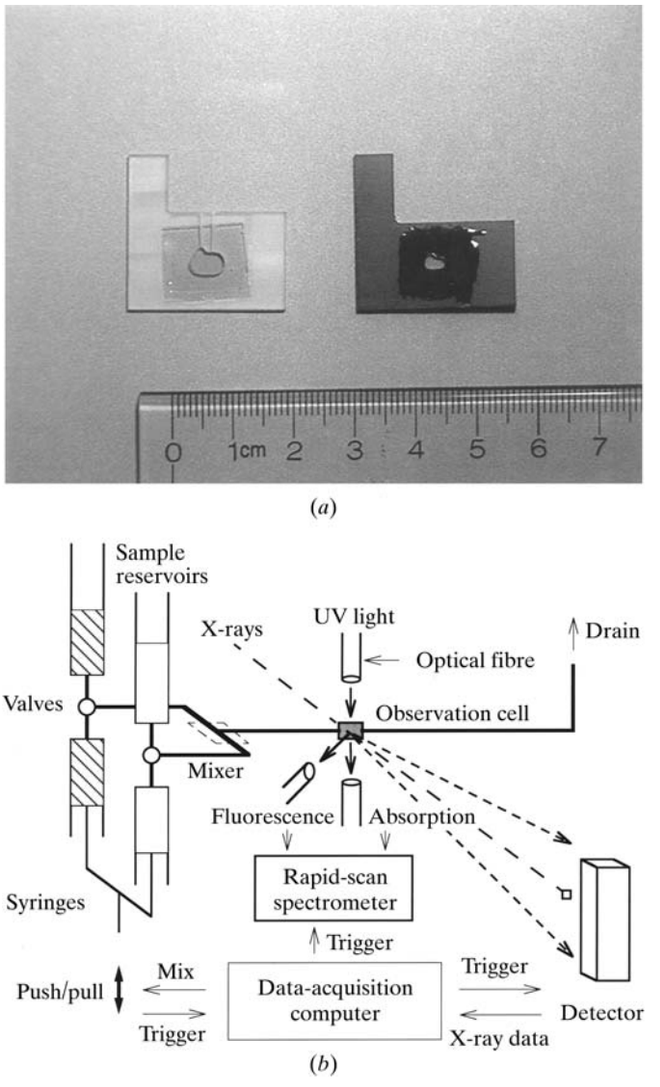

Fig. 19.3.3.6. (*a*) Flat-window cells for solution scattering and (*b*) a diagram of a stopped-flow rapid mixer for time-resolved solution scattering. The solution cell to the left in (*a*) is made of polycarbonate and is equipped with two synthetic mica windows. A sample solution is injected through one of two sample loading channels using a microsyringe. The black cell to the right is made of coloured polyoxymethylene for light-activated, time-resolved studies and has a smaller sample chamber. In (*b*), two solutions, *e.g.* an enzyme solution and a substrate solution, are put in sample reservoirs, loaded into individual syringes and wait for a trigger signal from the data-acquisition system. Then the two solutions are rapidly mixed, transferred to the observation cell, typically within 5 ms or less, and a trigger signal initiates a series of time-sliced scattering-data acquisitions. This stopped-flow mixer is also equipped with optical paths to monitor absorption or fluorescence from the protein solution in the observation cell.

references