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Pneumatic Micro-Sprayer for Millisecond Time Resolution in Cryo-Electron Microscopy

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Transient structural states of macromolecules such as ribosomes, ion channels, etc. can be viewed with electron microscopy by rapid quenching to liquid nitrogen temperatures. Time resolution in cryo-electron microscopy can be achieved by modifying the standard method of specimen blotting and plunge freezing to produce thin aqueous specimens on carbon-coated electron microscope grids. The key challenge of time-resolved cryo-EM (TREM) is to rapidly mix reactants and macromolecules just milliseconds prior to cryo-quenching in liquid ethane.

One method of TREM is to create a thin aqueous film by blotting a grid onto which has been deposited a 3-5 microliter drop of the macromolecule-containing solution and then spraying a fine mist containing one or more of the small-molecule reactants onto the grid as it is being plunged into liquid ethane. The use of a standard guillotine-type plunger with a glass atomizer was reported [1]. A more recent modification utilized a computer controlled stepper motor to plunge the specimen in combination with a 3-10 kv voltage applied to a fine capillary containing reactants to form an electro-spray mist on the grid [2].

In this work we have devised a small coaxial micro-sprayer which has the specimen-conserving efficiency of the electro-spray, but does not have the problem of clogging that plagues the electro spray capillary. Typically the commercially available atomizers use 100 microliters or more of solution per grid, which can be a problem when reactants are in short supply. A problem we have had with the electro-spray is that capillaries of 5-10 micron diameter produce good spray plumes, but rapidly clog up whereas larger capillaries do not clog but do not produce a suitable electrospray. Another problem is that the average size of the electrospray microdroplets tends to be severalfold smaller in diameter than the optimal which is 1-2 microns.

The core of the new device is a 0.004 inch (100 micron ID, 360 micron OD) capillary that is placed at the apex of a hollow plastic cone having an exit bore of 400 microns nm (Fig. 1). Pressurized air (30-50 psi) is applied to the inner volume of the cone and spray fluid is applied through the capillary at a rate of 2-30 microliters per second. Air escaping through the small space between the capillary and bore of the cone atomizes the sample fluid as it is expelled. The sprayer is mounted on the computer controlled apparatus designed by H. D. White for TREM [2]. This instrument provides us with numeric control of syringe pump rate, blotting times and coordination with rate-adjustable plunging. Also included with the TREM apparatus is a two-stage bubbler to humidify the air stream entering the sprayer. Tests were done by spraying a 2 mg/ml ferritin solution onto hydrophobic carbon/Formvar-coated grids. Preliminary results indicate that droplets from 2-12 microns in diameter can be obtained along with a few much larger drops (Fig. 2).

References

[1] J. Berriman et al. *Ultramicroscopy* 56(4) (1994) 241.
[2] H. D. White et al. *J. Struct. Biol.* 144 (2003) 246.
[3] Supported by NIH/NCRR Biomedical Research Technology Program Grant RR01219 (PI J.Frank).

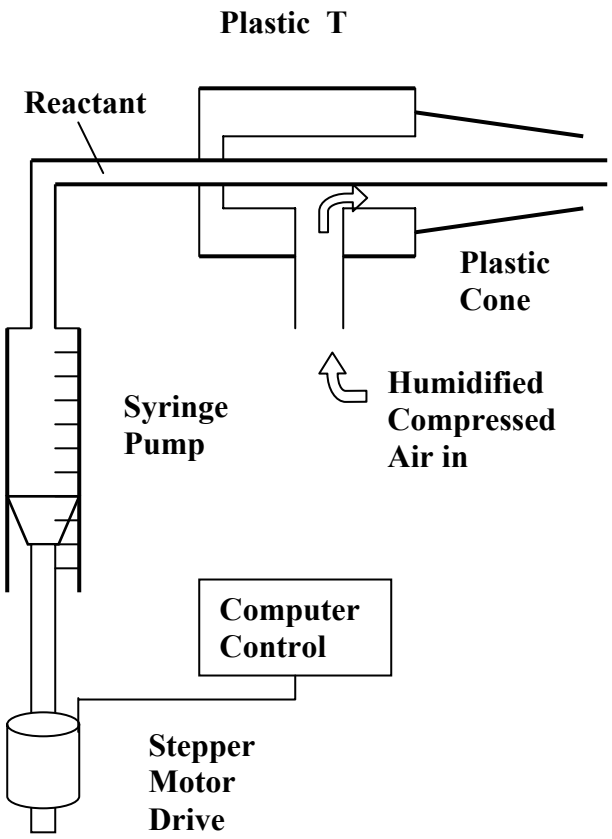

Fig. 1 Schematic Diagram of Sprayer

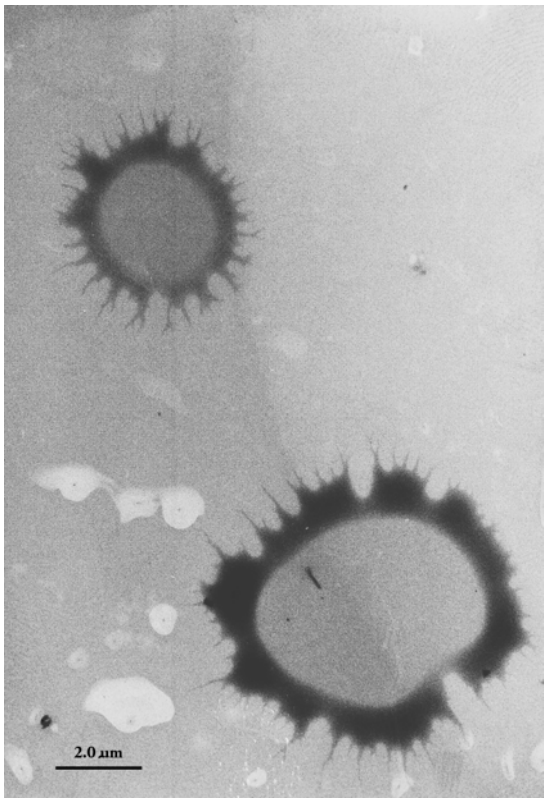

Fig. 2 Example of size of spray droplets deposited in grid. A 2 mg/ml solution of ferritin was sprayed at 3 micrograms /sec. as grid was plunged. Droplets were air dried.

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