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A Glow Discharge Unit to Render Electron Microscope Grids and Other Surfaces Hydrophilic

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ABSTRACT We describe the design, construction, and operation of a simple glow discharge unit that can be used to make surfaces such as carbon-coated electron microscopy grids and glass coverslips hydrophilic. The use of a vacuum leak detector (Tesla coil) in place of a conventional high-voltage power supply and a small plastic desiccator for the vacuum chamber make the unit very inexpensive. Owing to the small volume of the chamber and the simplicity of the unit, the whole glow discharge process can be carried out in only 2 to 3 min, a time considerably shorter than that required for conventional vacuum evaporators. The hydrophilic surface improves adsorption of particles by several orders of magnitude in preparation for negative staining, freeze-drying, and other procedures.

INTRODUCTION

Glow discharge in a reduced atmosphere of air has long been the accepted method to render carbon support films for electron microscopy (EM) hydrophilic (i.e., with a net negative charge) prior to adsorbing suspensions of biological material to them (cf. Dubochet et al., 1971). This is commonly performed in a vacuum evaporator by placing the EM grid between two parallel electrode plates to which a high voltage is applied to cause a glow discharge. We have found that it is faster and more convenient to employ instead a simple, custom-made glow discharge unit that is inexpensive enough for each investigator to have his own unit. Here we describe the design, parts list, construction, and operation of this glow discharge apparatus.

GENERAL DESCRIPTION

The vacuum-chamber is a plastic desiccator. The grids are placed on a grounded aluminum plate, and the chamber is partially evacuated (i.e., 10^{-1}–10^{-2} Torr) by a mechanical pump. A high-frequency vacuum tester (Tesla coil) attached to a second parallel aluminum plate (separated from the grounded plate by 20–30 mm) provides a high-voltage discharge of the residual air. The vacuum line to the pump is then closed by a needle valve, and air is admitted slowly to the chamber via a micrometric capillary valve (see Table 1).

CONSTRUCTION

The "top electrode" (G) consists of one of the round 100-mm-diameter aluminum plates (G) and is press-fit onto the 11-mm-diameter aluminum rod (J). The rod is fed through an 11-mm hole drilled in the top center of the polycarbonate upper section of the desiccator (A-1). The rod (J) is stabilized by a plexiglass washer (N) with an internal diameter of 11 mm and an outer diameter of 25 mm that is pushed into the neck of the cover. This feedthrough assembly is fastened to the cover and vacuum-sealed with epoxy glue.

To allow adjustment of the distance between the two parallel electrodes, the aluminium plate constituting the "bottom electrode" (H) is mounted on a threaded brass rod (K) that screws into a proper-sized nut (L) mounted around a central hole on the 140-

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TABLE 1. Parts list[1]

Part	Supplier	Catalog No.	Price
A. Plastic vacuum dessiccator (Bel Art), 149 mm diameter, 206 mm high	VWR[2]	24988-131	$24.00
B. High-frequency vacuum tester (Electro Technic Products)	VWR	63424-001	$89.00
C. Needle type valve (Nalgene), low-density polyethylene	VWR	65550-029	$9.00
D. Micrometric capillary valve (Gilmont)	VWR	65548-005	$71.50
E. Direct drive vacuum pump (Sargent Welch, Mod. 8803B)	VWR	4969-828	$310.00
F. Foreline trap (Sargent Welch, Mod. 1419A)	VWR	—	$323.00

[1]Construction also requires the following (see Figs. 1, 2): (G, H) two round aluminum plates (100 mm in diameter and 6 mm thick); (I) one round plexiglass plate 140 nm in diameter; (J) one aluminum rod 11 mm in diameter and 150 mm long; (K) one threaded brass rod 12.5 mm in diameter and 75 mm long with (L) a nut to be bolted in the center of the plexiglass plate; (M) copper ground wire; 1–2 m vacuum tubing; and two-component epoxy glue.
[2]VWR Scientific, P.O. Box 7900 San Francisco, CA 94120.

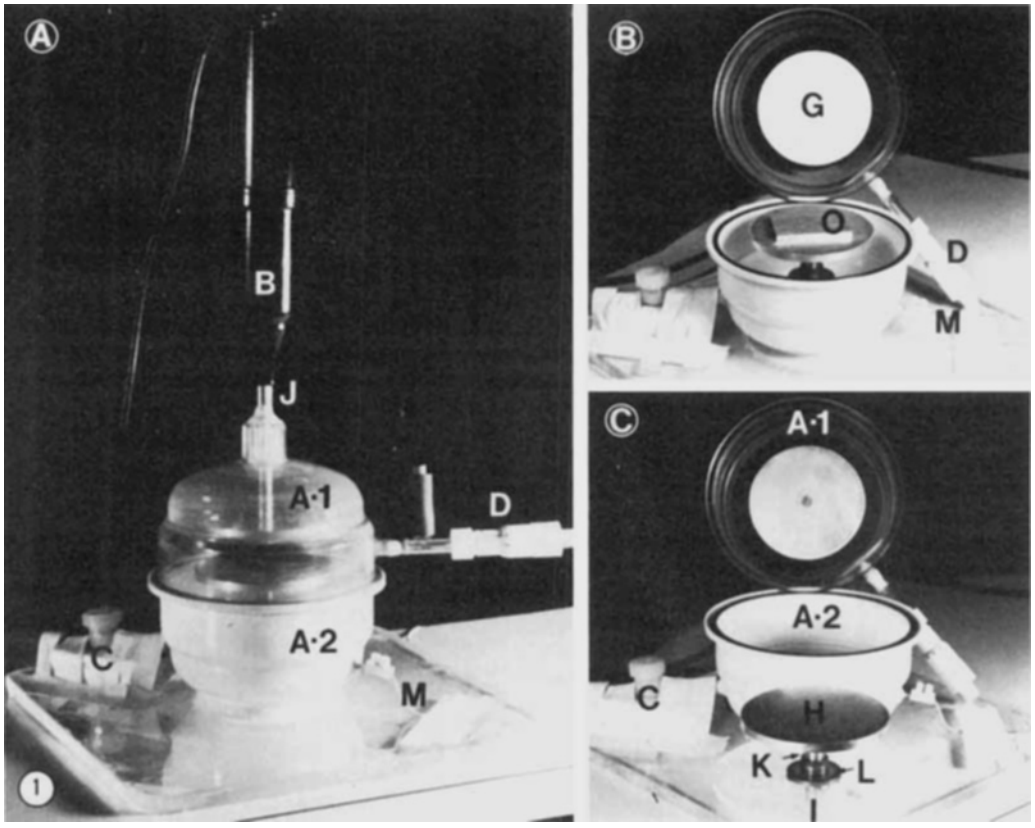

Fig. 1. Photographs of the glow discharge unit; **a,b,c** show various views. A-1 Upper section of dessicator; A-2, lower section of desiccator; B, high-frequency vacuum tester (Tesla coil); C, needle valve connecting chamber to vacuum line; D, micrometric capillary valve; G, upper electrode plate; H, lower, grounded electrode plate; I, plexiglass ground plate; J, rod connecting upper electrode to the Tesla coil; K, thread, adjustable support for lower electrode; L, nut; M, ground wire; N, plexiglass washer supporting electrode rod; O, grid carrier.

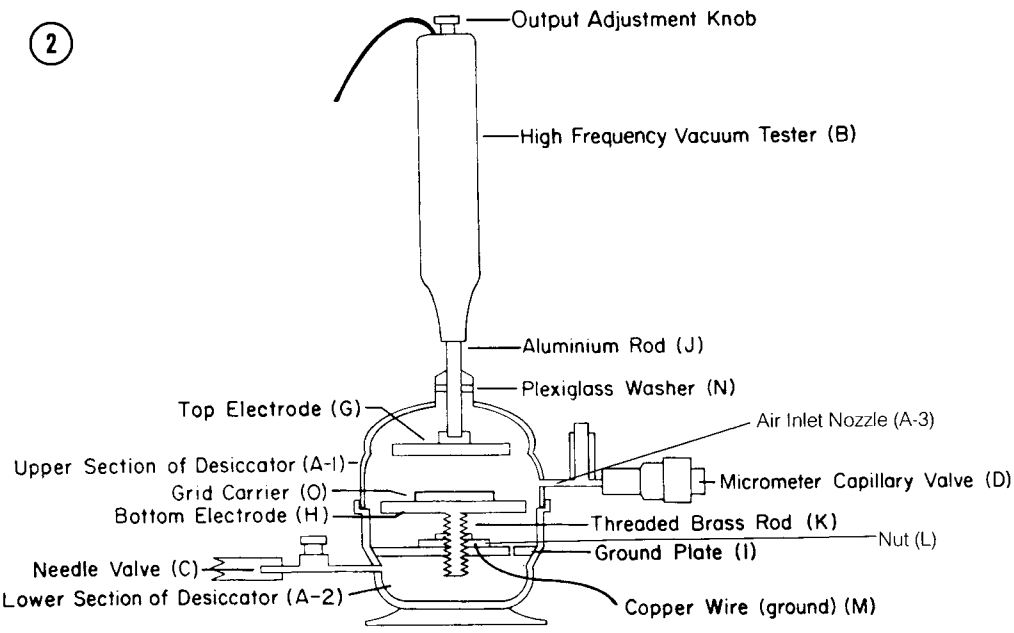

Fig. 2. Drawing of a central vertical section through the glow discharge unit. Same symbols as Figure 1.

mm-diameter plexiglass "ground plate" (I). A hole of about 5 mm in diameter is drilled through the ground plate to assure equalization of the pressure above and below the ground plate when in place inside the evacuated desiccator.

The needle-type valve (C) is glued into a 10-mm hole in the polypropylene lower section of the desiccator (A-2). The air inlet nozzle (A-3) in the polycarbonate upper section of the desiccator is drilled out to an inner diameter of 8 mm, and the micrometric capillary valve (D) is glued into the nozzle.

To ground the bottom electrode (H), a copper wire (M) is fed through the lower section of the desiccator (i.e., by heating the wire over a flame before pushing it through the polypropylene wall) and vacuum-sealed with epoxy glue. The copper wire is connected to the bottom electrode by soldering it to the nut (L) fastened to the ground plate. Good grounding is important for proper glow discharge!

Finally, it is necessary to remove the electrode tip supplied with the high-frequency vacuum tester (B) and connect the tester to the top of the 11-mm-diameter aluminum rod (J).

We use a simple direct drive vacuum pump to evacuate the chamber, but any available mechanical pump can be used. To minimize backstreaming of oil vapour from the pump, a molecular sieve type of foreline trap should be put into the vacuum line between the pump and the vacuum chamber.

OPERATION

Initial conditions are the following: Chamber at atmospheric pressure, needle-type valve (C) closed, and pump off. EM grids (or other objects to be treated such as glass coverslips) are placed on a rectangular (40 mm × 60 mm), 5-mm-thick aluminium grid carrier (O), scribed with one or more grooves to facilitate picking up the grids. While the upper section of the vacuum chamber (A1) is lifted up, the grid carrier is put onto the bottom electrode (H); then the upper section is put back in place, and the micrometer capillary valve (D) is closed. Next, the pump is turned on, and after about 5 sec (to allow evacuation of the line between the pump and the vacuum chamber), the needle-valve (C) is opened to evacuate the chamber. After the pressure has reached 10^{-1}–10^{-2} Torr (which is after 1–2 min with the mechanical pump listed above; see Table 1), the vacuum tester is turned on with the output set with the adjustment knob to generate an intense purple-to-violet (i.e. ozone generating) glow dis-

charge between the two parallel electrodes. The duration of the discharge is adjusted depending on the application. For carbon films on EM grids, 5–15 sec yields a surface that will adsorb an even distribution of particles during a 30–60-sec application of a 2.5-μl drop of material having a particle concentration of 10–50 nM. After glow discharge is completed, the needle-valve is closed, and air is admitted slowly (to prevent the grids from being blown away!) to the chamber through the micrometric capillary valve (D). Finally, the grid carrier is removed, the rotary pump is switched off, and the needle-valve is opened to ventilate the line between the pump and the vacuum-chamber (i.e. to prevent oil from backstreaming into the vacuum line). The whole cycle of operation takes only 2–3 min.

EVALUATION

We have used this unit successfully for over 6 years to render carbon-coated EM grids hydrophilic for both negative staining and adsorption freeze-drying (cf. Kistler et al., 1977) of molecular and supramolecular suspensions. The desired effect is to make the surface of the specimen support film sufficiently charged that a thin aqueous film will spread evenly and dry down evenly over the entire surface, even when the bulk of the liquid is removed by touching a piece of filter paper to one edge. With support films treated in the glow discharge device, the concentration of macromolecular suspensions can typically be lower by 1–3 orders of magnitude compared with the concentration needed for sufficient adsorption to untreated films.

We find that this simple device is just as effective, but faster and considerably less expensive, than commercially available vacuum evaporators. Some of the applications have included preparation (i.e. by negative staining and/or freeze-drying/metal-shadowing) of unaggregated actin filaments and actin filament paracrystals (Fowler and Aebi, 1982, 1983; Smith et al., 1984), crystalline actin sheets (Aebi et al., 1980, 1981), myosin filaments (Pollard, 1982), bundles of actin filaments from microvilli (Pollard and Mooseker, 1981), intermediate-sized filaments (Aebi et al., 1983), and crystalline arrays of Ca^{2+}-ATPase (Buhle et al., 1983).

The glow discharge unit can be used to put either a positive or negative net charge on a surface. While in a reduced atmosphere of air, glow discharged carbon support films have a net negative charge; glow discharge in a reduced atmosphere of pentylamine results in a net positive charge of carbon support films (cf. Dubochet et al., 1971). This becomes particularly useful when trying to achieve protein-free (i.e., without cytochrome C) spreading of DNA such as in situations in which one wants to visualize or map proteins (e.g., RNA polymerase, repressors, restriction endonucleases, etc.) bound to DNA (cf. Williams, 1977; Brack and Pirotta, 1975; Brack et al., 1976). Such positively charged support films may easily be prepared with our glow discharge apparatus, simply by connecting the air inlet nozzle of the micrometric capillary valve to a bottle of pentylamine and adjusting the pentylamine vapour pressure in the vacuum-chamber with the capillary valve such that an intense violet-to-blue (i.e. versus purple-to-violet in a reduced atmosphere of air; see above) glow discharge between the two parallel electrodes is obtained. It should be stressed, however, that pentylamine slowly but definitely attacks the polycarbonate from which the upper section of the desiccator used here is made. If glow discharge in pentylamine will be routinely employed, the chamber should be constructed from a glass desiccator.

Earnshaw and Migeon (1985) have discovered that glass coverslips treated in the glow discharge unit can be used to obtain superior spreads of chromosomes for light microscopy. This expands considerably the potential applications for the glow discharge process to disciplines in which laboratories are not equipped with vacuum evaporators and in which a simple, custom-made glow discharge unit may be particularly desirable.

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