Metallic Conical Wells for Containing and Transferring Pollen for Scanning Electron Microscopy

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We have increased the efficiency for containing and transferring pollen during chemical drying procedures for conventional scanning electron microscope (CSEM) viewing by replacing standard laboratory containers (glass test tubes, pipettes, etc.) in the final drying stage with smoothed and polished metallic conical wells. The wells, formed by drilling into one end of aluminum CSEM specimen holders, are approximately 4 mm deep, 8 mm in top diameter, and 1 mm in bottom diameter. The large surface area allows rapid elimination of dehydrant. Unlike standard containers, to which dry pollen sticks after evaporation or sublimation of dehydrant, the wells allow pollen to be easily collected, concentrated, and transferred to CSEM specimen holders with minimal loss of sample. The conical wells have been used with alcohol, Peldri-II, and hexamethyldisilazane drying procedures and therefore, universal application seems likely.

INTRODUCTION

Pollen must be dry before it can be examined in a conventional scanning electron microscope (CSEM). The simplest way to do this is by air-drying cleaned (*1*) and/or treated (*2*) pollen directly from absolute ethanol (EtOH) onto specimen holders, after the pollen has passed through a graded series of (EtOH-H_2O) solutions with ascending concentrations of EtOH. This approach is expedient and insures minimal loss of pollen during dehydration and transfer to specimen holders, but it is often unacceptable because physical drying stresses can cause the pollen to wrinkle or collapse. In addition, the pollen grains are often poorly attached to the specimen holders and may be dislodged before CSEM viewing; they may also be so widely dispersed that they are difficult or impossible to locate; cf. Fig. 1, bottom inset (*6*).

Other drying procedures employed in conventional scanning electron microscopy [critical point drying (CPD; *3*), freeze drying (FD; *4*), Peldri-II drying (PD; *5*), or hexamethyldisilazane drying (HMDS; *6*)] provide better preservation of pollen structure. However, all these procedures require a container, usually a glass pipette or test tube, to complete the drying process (for example, Fig. 1H of reference *5*). Prior to examination in the CSEM, chemically dried pollen is transferred to CSEM specimen holders by first tapping the containers to loosen the pollen and then sprinkling the free grains onto a holder, usually covered with an adhesive (Fig. 1I of reference *5;7*). Frequently, some of the dry pollen adheres to the sides of the containers (Fig. 1, top and middle insets) and an appreciable amount is lost in the transfer process, as discussed in depth elsewhere (*5*). Additionally, drying in glass test tubes or pipettes requires extended time periods because the long side walls and small openings retard speedy elimination of dehydrant (for example, evaporation of EtOH and HMDS, and sublimation with PD; Fig. 1, top and middle insets).

METHOD

We have improved the speed of drying and reduced the amount of pollen lost during transfer by performing the final drying step in shallow, cone-shaped wells that have been drilled into one end of JSM-2 (JEOL Inc.) cylindrical aluminum CSEM specimen holders (10 × 10 mm), using a 3/8-inch drill bit with a 110° tip. The specimen holders are readily available through commercial electron microscopy supply dealers. The wells are approximately 8 mm in diameter at the top, 4 mm in depth, 1 mm in diameter at the base, and highly polished with metal polish after initial smoothing with steel wool (Fig. 1A). The wide top diameter allows rapid elimination of dehydrant, and the conical shape concentrates the pollen at the well bottom (Figs. 1B-C). The highly pol-

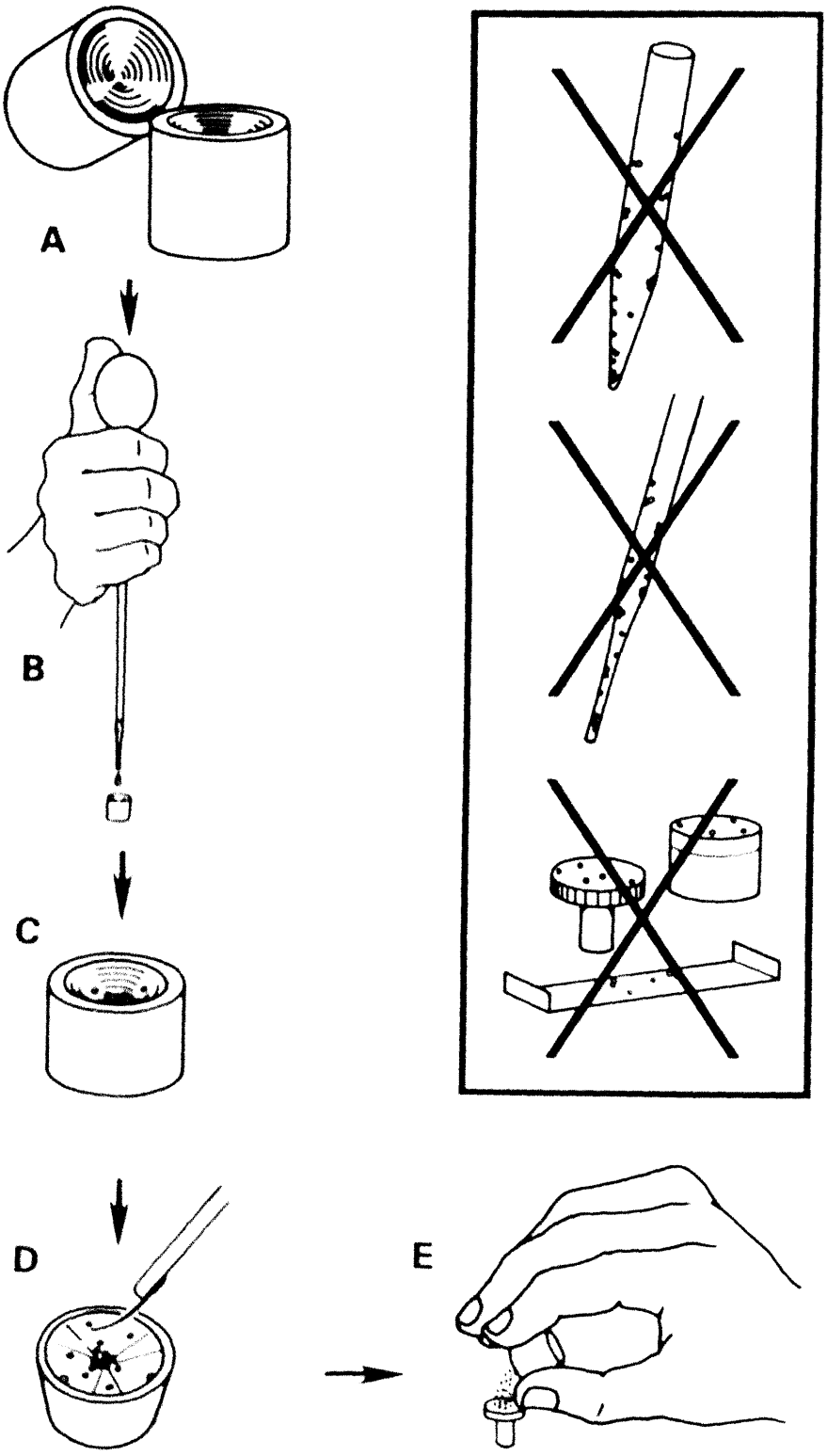
A
B
C
D
E

Figure 1, Parts A-E. Conical wells and their use in preparing pollen for CSEM.

Part A. Diagrams of conical wells made from drilled-out CSEM specimen holders.

Part B. Pollen in dehydration fluid (EtOH, Peldri-II, HMDS**,** etc.) is dripped from pipette into conical well.

Part C. After evaporation of dehydrant, dry pollen is dispersed on the sides and bottom of the well. The large surface area enhances speed of pollen drying and increases the number of well-preserved grains.

Part D. Pollen is concentrated in well bottom by freeing adherent grains from highly polished well with an eyelash stick. In order to obtain maximum transfer of pollen, we routinely brush the entire well area to free the pollen and then gently "tap" the well to concentrate the sample. This procedure, in conjunction with the highly polished surface, guarantees essentially 100% transfer of pollen without typical loss due to sticking to side walls.

Part E. The concentrated and loosened pollen is transferred to a CSEM specimen holder covered with an adhesive [not shown in diagram but consisting of carbon tape, Tempfix (*7*), silver paste, etc.].

Inset: The X's crossed through each figure summarize some problems encountered when transferring dry pollen using standard laboratory containers. Top and middle insets- with test tubes and pipettes, drying is less than desirable because: (a) fumes released from dehydrant must travel considerable distances along the sides of the glass tubes, which markedly increases drying time, and (b) dry pollen is difficult to free from the sides of the glass vessels. Bottom inset - when air or chemical drying is done directly on CSEM specimen holders pollen is frequently highly dispersed and difficult to locate with the CSEM and, as discussed elsewhere (*6*), often unstable.

ished finish promotes nearly total pollen transfer to CSEM specimen holders because dried pollen grains adhering to the interior surface of the well can be easily loosened with an "eyelash" stick (an eyelash glued to a thin wood stick) or fine brush (Figs. 1 D-E).

It is less important to emulate the exact dimensions of the wells than it is to insure a well that provides a large surface area, cone-shaped sidewalls, short depth, small bottom diameter, and highly polished surface. Although we used wells made of aluminum, any metal, such as copper, brass, or stainless steel, is suitable. Similarly, CSEM specimen holders can be of other brands and dimensions; they can also be made from any round metallic rod stock. The important point is that the well should not be plastic as dehydration fluids can react with the synthetic organic components and contaminate the sample. Attainment of these conditions will enable dehydrants to escape rapidly as well as allow direct access to the dried pollen. We have used conical wells with EtOH, HMDS and PD drying and feel that they can be used in other drying procedures. Further, the shallow wells may be suitable for manipulating pollen samples that are sparse, including single pollen grains and spores frequently encountered in geological preparations. Our procedure for drying pollen in conical wells is outlined in Figure 1.

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