



FIGURE 3 Scanning electron micrographs (SEMs) of 10% hydroxyethyl starch (HES) frozen in a vial by liquid nitrogen immersion. (A, B) Top of the cake (A not annealed, B annealed); (C, D) interior of broken cake (C not annealed, D annealed). *Source:* From Ref. 4.

FREEZING METHODS

Various freezing methods can be used for liquids in glass vials, although some are not appropriate for full-scale GMP production of sterile pharmaceuticals. Principal methods are listed below in order of increasing cooling rate:

1. Slow directional solidification: creating ice nuclei on the bottom of a vial by contact with dry ice, followed by slow freezing on a precooled shelf (9,10)
2. Vacuum-induced freezing (11)
3. Placing vials on a shelf which is then ramped from above freezing temperature to below T_g' ("shelf-ramp" freezing)
4. Placing vials in a freezer or on a lyophilizer shelf which is already below freezing temperature ("precooled shelf" freezing)
5. Immersion in refrigerated heat transfer fluid (e.g., dry ice in alcohol)
6. Blast freezing via forced air and/or sprayed liquid nitrogen (12)
7. Liquid nitrogen immersion freezing

By far the most common method is the third one listed—shelf-ramp freezing. There are no issues with condensation on the shelves during loading, and all lyophilizers can carry out this type of freezing without modification.

Specific ice nucleation techniques have been used for various purposes. Examples include nucleating agents silver iodide and *Pseudomonas syringae* (4),