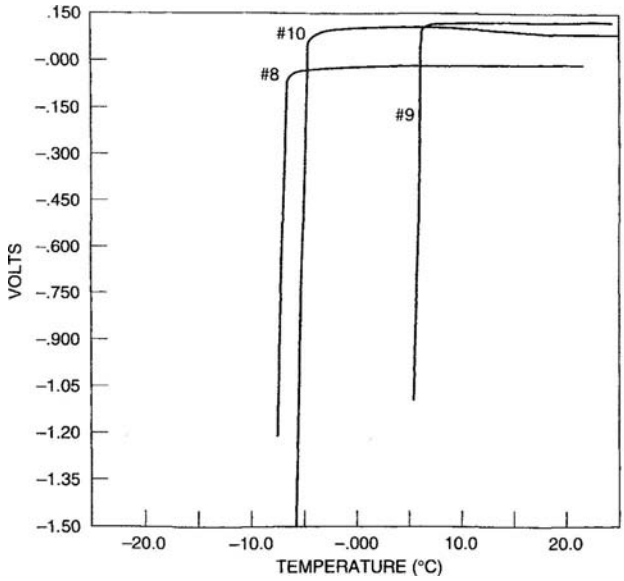

FIGURE 21 Vapor pressure moisture condensation temperature curves for three lots (8, 9, and 10) of U.S. standard pertussis vaccine.

time. This vapor pressure moisture methodology is being applied to the study of vial-to-vial variability within one lot and redistribution of moisture between cake, headspace, and stopper or headspace and cake over time.

Methodology in Use by Manufacturers of Biological Products

The methods approved for analyzing residual moisture in freeze-dried biological products licensed by the FDA are the gravimetric (loss-on-drying) method, many variations of the Karl Fischer method, TG, gas chromatography, and a modification of the moisture evolution analyzer (MEA).

Gravimetric Methods

Flosdorf (1) described the gravimetric method in 1949. There are several variations on the basic gravimetric method in use. The test may be carried out in a relatively large dry box rather than in the humidity-controlled room. The sample may be heated in a drying oven. The Abderhalden method uses a small sample size and heat is provided by a refluxing solvent. The sample is dried in the Abderhalden apparatus over refluxing liquid that boils near room temperature. There are other variations in terms of time, temperature, and vacuum that have been licensed for a particular product since, on a case-by-case basis, data demonstrated that at a chosen temperature a constant weight loss was obtained without decomposing the product.

Karl Fischer Methods

The approved variations (17) in the Karl Fischer method include volumetric titration methods to either a visual (excess iodine or addition of an indicator) or