

# Regulatory Control of Freeze-Dried Products: Importance and Evaluation of Residual Moisture

Joan C. May

*Center for Biologics Evaluation and Research, United States Food and Drug Administration, Rockville, Maryland, U.S.A.*

## INTRODUCTION

The residual moisture content of the freeze-dried biological product is usually near 1% (wt/wt) to 3% (wt/wt). Optimum residual moisture limits are set for each freeze-dried biological product on a case-by-case basis. Residual moisture limits for the freeze-dried product are supported with stability data that demonstrate that at the recommended moisture level the safety, purity, and potency of the product is maintained throughout the product's dating period. The Center for Biologics Evaluation and Research (CBER) of the U.S. Food and Drug Administration (FDA) regulates freeze-dried biological products in its section pertaining to residual moisture, as published in Title 21 of the Code of Federal Regulations for Food and Drugs. The regulation requires that each lot of dried product be tested for residual moisture and meet and not exceed established limits as specified by an approved method on file in the product license application. Specific information about test methods and illustrative residual moisture limits for vaccines and biological product is addressed in the CBER Guideline for the Determination of Residual Moisture in Dried Biological Products. Many recent articles report studies that have focused on residual moisture as it relates to potency, formulation, aggregation, and container closure and stopper considerations. Methods used for the measurement of residual moisture in the freeze-dried final container include the traditional loss on drying, Karl Fischer, and gas chromatographic methods as well as recent advances in coulometric Karl Fischer methods, thermogravimetry (TG), and thermogravimetry/mass spectrometry (TG/MS). Special applications for near-infrared reflectance (NIR) spectroscopy and tritium isotope methods are described. Recent advances in vial headspace moisture methodology (vapor pressure moisture) research have the potential to shed light on moisture and processes occurring within the freeze-dried vial over time, for example, redistribution of moisture between container closure, container headspace, and freeze-dried cake. In addition, there is the redistribution of moisture between surface moisture and bound water in the various chemical constituents such as protein and hydrated salts in the freeze-dried cake.

## Residual Moisture

Residual moisture is the low level of water, usually in the range of less than 1% to 3% (wt/wt), remaining in a freeze-dried product after the freeze-drying (vacuum sublimation) process (1–8) is complete. Nail and Johnson (9) have described in-process methods to monitor the endpoint of freeze-drying using residual gas analysis, pressure rise, comparative pressure measurement, and