denaturation of the I_gG at the ice/freeze concentrate interface that is reversible upon freeze-thawing, but becomes irreversible after freeze-drying and reconstitution. Breen et al. (43) studied the effect of residual moisture in the range of 1% to 8% on the stability of a lyophilized humanized monoclonal antibody formulation. They found that high-moisture cakes had higher aggregation rates than drier samples if stored above their T_g values. Intermediate moisture-containing cakes were more stable to aggregation than low-moisture-containing cakes.

Container Closure/Stoppers and Residual Moisture

Pikal and Shah (44) studied moisture transfer from stopper to product and the resulting stability implications. They found that the product moisture content increases with time and reaches an apparent equilibrium value characteristic of the product, the amount of product, and the stopper treatment method. Templeton et al. (45) and Donovan et al. (46) conducted further studies relating stoppers, stopper processing, and product final moisture content.

Crist (47) studied lyophilized pharmaceuticals sealed under reduced pressure and demonstrated that vial pressure is primarily due to desorption of water vapor from the stopper into the headspace of the vial. The presence of a hydrophilic product decreases the rate of pressure rise, and pressure increases more rapidly in smaller vials. Results are discussed in terms of testing for seal integrity and for stopper effects on moisture in the product.

Pikal and Shah (48) studied intravial distribution of residual moisture in dextran, human serum albumin, and bovine somatotropin. In general, the residual moisture in the top of a core sample of the freeze-dried product was less than the moisture content in the bottom core section of the product. The section closest to the vial wall was consistently found to be lowest in moisture content.

RESIDUAL MOISTURE MEASUREMENT IN THE FREEZE-DRIED FINAL CONTAINER

Current Methods

The methods for the determination of residual moisture currently used at the CBER at the FDA are the gravimetric (loss-on-drying) method, the Karl Fischer method, and the TG and TG/MS method. Current work in progress involves the use of vapor pressure moisture measurements to provide additional information about residual moisture content and its interaction with the components of the freeze-dried final container and its contents.

Gravimetric Method (*Loss on Drying*)

The gravimetric method measures surface moisture and loosely bound water of hydration (20). The gravimetric method was described in the U.S. Code of Federal Regulations (18) before 1990. In the CBER/FDA laboratory, the gravimetric (loss on drying) test (2,49) is performed in a humidity- and temperature-controlled room. In actuality the room is a walk-in incubator converted to maintain the 20% to 25% relative humidity range and 20°C to 25°C temperature range required by the test. The room contains a five-place analytical balance on a marble balance table, vacuum pump, Pirani vacuum gauge, hygrometer, desiccators, and sulfuric acid scrubbers for the air used to release desiccator