Freeze-dried cakes – Where is the water?

Isobel Cook¹, Kevin Ward¹, Derek Duncan²

¹Biopharma Technology Ltd, Winchester, UK, ²Lighthouse Instruments LLC, Charlottesville, USA

Introduction

Water may be present in a variety of ‘forms’ – free, adsorbed, chemically bound, crystallized or associated (e.g. of protein). Water of crystallisation, not all of which may be directly linked to the activity or stability of the product in question. Currently the best established water detection methods, Karl Fischer (KF) titration and thermo-gravimetric analysis (TGA) do not necessarily allow the operator to distinguish between one form of water and another. In this study, a number of freeze dried cakes have been analysed, ranging from purely amorphous to completely crystalline, using KF and FMS (frequency modulation spectroscopy) KF titration is a well established and FDA recognised technique for moisture analysis and is generally considered to measure the total water within the vial containing the freeze dried cake, if the sample is wholly soluble in KF medium. FMS analysis is a relatively new technique that measures the moisture and pressure in the vial headspace and is a non-destructive technique, allowing the same sample to be monitored over time. The aim of the present study is to understand the relationship between the moisture measured by KF/FMS and further our understanding of product-water interactions within a freeze dried cake.

Materials and Methods

Coulometric KF (Cou-lo Aquamass, GRS Scientific) was used to measure the total water within the freeze dried cake while FMS (FMS-1400, Lighthouse Instruments) enabled the measurement of water pressure and moisture within the headspace of the vial. The laser is tuned to match the internal absorption frequency of a water molecule and the amount of laser light absorbed is proportional to the water vapour concentration and the absorption width of the signal is proportional to the pressure. In order to obtain good freeze dried cakes the temperature of the pre-lyophilised material was measured using a freeze drying microscope Lyostat2 (BTU) and crystallisation/mobility changes were studied using a thermal/impedance analyser, Lytherm2 (BTL). A number of excipients (mannitol, glucose, trehalose, PEG, BSA, NaCl, sucrose, KCl) were analysed following a series of freeze drying cycles and monitored over time. Experiments were purchased from Sigma-Aldrich and BDH. The same vial and stopper types (not oven dried unless stated) were used; vials were sealed under a pressure of 50mTorr. KF measurements were taken once long term monitoring showed FMS readings had become constant.

Results and discussion

KF/FMS Linearity – Experiments conducted to date indicate that there is an observable relationship between KF and FMS results. The following graph (Fig 3) shows the correlation for sucrose with an R² of 0.9225. The best fit line is straight through the origin, but instead, the value of the intercept may be a reflection of the nature of the water present, which in turn may be a function of the state of the excipients. For example, if a freeze-dried material (e.g. sucrose) harbours tightly associated water, this may only be detected by KF and not FMS, which measures only equilibrium free water.

Temperature effect – Headspace moisture was studied for several excipients at 8°C, 25°C and ambient (20 ± 1°C). Samples were monitored every 30 seconds for up to an hour as the temperature naturally returned to ambient conditions. A blank trial was performed on a reference standard (no cake, no stopper, glass sealed) and an empty vial (no cake, glass vial, stopper) to assess whether any variations in measurement were due to other effects (e.g. condensation on removal of a sample from a 2°C/30°C moisture variation test – temperature). The reference standard did not show any significant moisture variation, however temperature had a significant effect on the empty vial (data not shown). This indicates that the stopper/headspace equilibrium moisture is highly sensitive to temperature, which is supported by Donovan (2007). Reference standards showed experimental readings of the water present in lyophilised products and establishes the importance of gaining a thorough understanding of the state of the excipients. For example, if a freeze-dried material contains a high level of moisture this may be directly linked to the activity or stability of the product in question. The sensitivity to temperature changes over time – mannitol 2% wt (product A) and mannitol 2% + glucose 1% wt (product B) were from the same freeze drying run with the same type of vial and stopper, samples were analysed within 1 hour of each other. Ambient temperature differences account for some of the fluctuations; however, long term trends are not a result of temperature - the crystalline excipients show a noticeable moisture (P/M) ratio further assists in the interpretation of the data. Product B shows an overall decrease in the P/M ratio over time; see Fig 6 indicating more moisture has entered the headspace; whilst product A has a constant ratio. These differences could be due to a number of factors, such as the ingress of moisture from the stopper, the ability of the samples to take up water, the uncertainty of the water present in lyophilised products and establishes the importance of gaining a thorough understanding of the state of the excipients. For example, if a freeze-dried material contains a high level of moisture this may be directly linked to the activity or stability of the product in question. The sensitivity to temperature

Conclusions

The data presented here provide evidence that using FMS as a complementary method to KF may enable the elucidation of the data and dynamics of water present in lyophilised cakes. It has been possible to investigate changes within a series of samples and assess how they are related to amorphous/crystalline changes, as well as headspace moisture resulting from stoppers and temperature effects. This study demonstrates the complexities involved in understanding the effect of the water present in lyophilised products and establishes the importance of gaining a thorough understanding of the excipients, the various process conditions, temperature, storage, and stopper properties in order to understand and evaluate results for repeatable and accurate FMS analysis. A water content Imbalance of 15mg could be significant, especially in low solid dose products. The rate of this moisture increase will also affect whether current KF analysis procedures already account for this due to the time delay between production and KF analysis. The importance of storage temperature is reiterated here as it will affect the amount of water resident in the cake, which may cause unwanted physico-chemical changes. Once headspace moisture studies have been more thoroughly investigated and understood, this method could be applied to the non-intrusive and rapid analysis allowing the monitoring of large production batches, possibly on a 100% inspection basis.

Further work

It is acknowledged that a greater range of KF and FMS results are needed for further study on observed differences. Linearity and point of intercept is of particular interest and could yield important information. A number of factors such as the variation points to be correlated, protein denaturation shells and mannitol polymorphs may affect water within the cake and the intercept could provide information on this. Fill depth and exipient concentration will also affect the ratio and these effects also need to be assessed. A closer study on temperature and headspace moisture is also warranted to further understand the relationship between these parameters. The temperature tightly controlled, chemical and physical changes could be more accurately monitored and predicted over time. Lighthouse Instruments has developed a temperature controlled model and further work is intended with this.

References