Freeze dried cakes – Where is the water?

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Introduction

Water may be present in a variety of "forms" – free, adsorbed, chemically bound, hydration shells (e.g. of proteins), 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 total moisture analysis and 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 Aquamax, GRS Scientific) was used to measure the total water within the freeze dried cake while FMS (FMS-1400, Lighthouse Instruments) enables the measurement of water and pressure 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 collapse temperature of the pre-lyophilised material was measured using a freeze drying microscope, *Lyostat2* (BTL) and crystallisation/mobility changes were studied using a thermal/impedance analyser, *Lyotherm2* (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. Excipients 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

FMS standard data - Moisture standards showed experimental readings consistent with reference values to provide a standard curve between 0.5 Torr and 9.9 Torr (r^2 = 0.9999, see Fig 1) with individual standards also displaying consistent readings over several months (Fig 2).

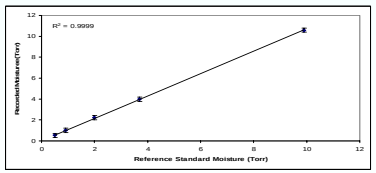

Figure 1 – Moisture reference standard values against the average measured value with standard deviation error bars

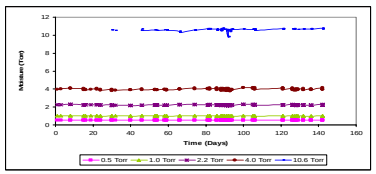

Figure 2 - Standard moisture values recorded over time

KF/FMS Linearity – Excipients studied 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 r^2 of 0.9225. The best fit line does not pass through the origin, but instead, the value of the intercept (c) 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".

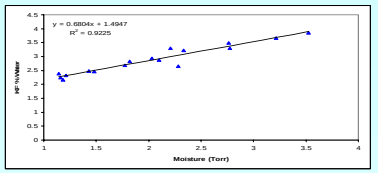

Figure 3 – KF/FMS correlation

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 8°C/stopper moisture variation with 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, this is supported by Donovan et al (1). The data in Fig 4 shows that temperature has a significant impact on the moisture within the headspace; this pattern has been observed in both amorphous (BSA) and crystalline (mannitol) excipients. Temperature is an important factor affecting the stopper moisture equilibrium and additionally the level of moisture either available or already within the cake; the excipients play an important role in the transfer and magnitude of the observed changes.

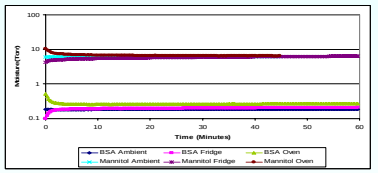

Figure 4 – Moisture response to temperature

Stopper moisture contribution – Stoppers can be dried to remove residual moisture prior to freeze drying. The average loss on drying value for the butyl stoppers was 0.5mg after 3hrs at 105°C. In this study we compared dried and un-dried stoppers and then measured the effect on headspace moisture. An increase in headspace moisture is clearly evident by FMS, demonstrating the ingress of moisture from the stopper when stoppers are not oven dried prior to freeze drying. Importantly, the amorphous and crystalline content within the vial results in a marked difference in the observed increase in headspace moisture, see Fig 5 with mannitol (crystalline) and sucrose (amorphous). A small initial decrease in the headspace moisture for mannitol is also evident; this may be due to uptake of free water by the cake, possibly to effect a polymorphic change.

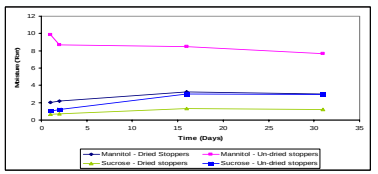

Figure 5 – Stopper moisture

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 types 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 and the use of the pressure/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, surface area and conceivably physico-chemical stability of the samples, all of these affecting the moisture equilibrium reached and its sensitivity to change. Another point to be considered is that product B has undergone a gradual crystallisation of any amorphous mannitol; this is supported by evidence from the freeze drying microscope and thermal analyser indicating mannitol crystallisation is retarded by amorphous glucose.

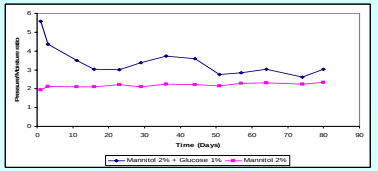

Figure 6 – P/M ratio over time

Excipient effects on KF and FMS ratios – Table 1 shows KF, FMS and KF:FMS ratios for a range of crystalline and amorphous materials and combinations thereof. These data suggest different proportions of water in the cake and the headspace that may be attributed to the degree of crystallinity in the cake as well as the hydroscopic nature of the cake. A higher KF:FMS ratio implies a higher proportion of total water being resident in the cake. The two amorphous excipients monitored have a much higher ratio than the crystalline excipients. NaCl has an especially low ratio, which could be due to its crystal form, as above 1°C the di-hydrate converts to anhydrous NaCl, (see Franks and Auffret (2)). Since anhydrous NaCl contains no structural water, all the water present within the cake should theoretically be in equilibrium with the headspace moisture. KF:FMS ratios could be affected by the physico-chemical form (e.g. anhydrous, hydrate, polymorphic state), ability of samples to take up water, condition of the cake (e.g. micro-collapsed, partially collapsed, collapsed) density/surface area, fill depth, as well as environmental conditions (e.g. temperature, stopper moisture equilibrium, sample processing conditions).

	Sucrose 4%	Mannitol 2% + Glucose 1%	Mannitol 2%	NaCl 5%
Karl Fischer % water	2.87	3.9	5.04	1.03
FMS Headspace Moisture (Torr)	2.03	3.3	6.32	6.13
KF: FMS Ratio	1.39	1.2	0.79	0.17

Table 1 - Table of KF and FMS results

Conclusions

The data presented here provide evidence that using FMS as a complementary method to KF may enable the elucidation of the location 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 vial stoppers and temperature effects. This study demonstrates the complexities involved in understanding the 'nature' 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 increase of 0.5mg 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 moistures 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 various crystalline hydrates, protein hydration shells and mannitol polymorphs may affect water within the cake and the intercept could provide information on this. Fill depth and excipient 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 location and dynamics of the water within the system. Once constant parameters have been established and 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

1. Donovan et al, PDA J Pharm Sci Technol. 2007 Jan-Feb;61(1):51-8
2. Franks and Auffret, Freeze-drying of Pharmaceuticals and Biopharmaceuticals,RSC, ISBN-13: 978-0-85404-268-5