

A study of vial headspace moisture in an entire freeze dried batch and the factors affecting moisture content variability

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Contents

- Brief outline of the basics of the freeze drying process
- Importance of moisture and analysis method
- Introduction to headspace moisture analysis
- Correlation of vial headspace moisture and Karl Fischer moisture
- Moisture mapping results
- What headspace moisture can tell us
- Summary

The freeze drying basics

Why - Freeze drying can increase product shelf life from a few days to months/years.

Involves removing the water from the material which involves three stages

- **Freezing** - freeze the product to below its critical temperature
- **Primary drying** - remove the free ice by sublimation under vacuum
- **Secondary drying** - remove any remaining unfrozen water, the temperature is typically raised above 0 C and the vacuum increased

For successful freeze drying

- **Choose suitable components for the formulation**
- **Establish the critical temperatures for the product**



Liquid fill

Freeze
dried
→



Good cake



Collapsed cake

Moisture in the freeze dried product

The moisture content within a freeze dried material has a direct effect on the glass transition (T_g) of the material. The T_g is the point at which a material can be observed to undergo structural change, this has a direct effect on the

- **Long term stability**
- **Storage temperature**

Establishing moisture content uniformity is an important quality control tool

Moisture is commonly measured by Karl Fischer (KF) analysis and is often considered the industry standard

- **Measure the total water**
- **Labour intensive**
- **Destroy the sample**
- **Toxic reagents**



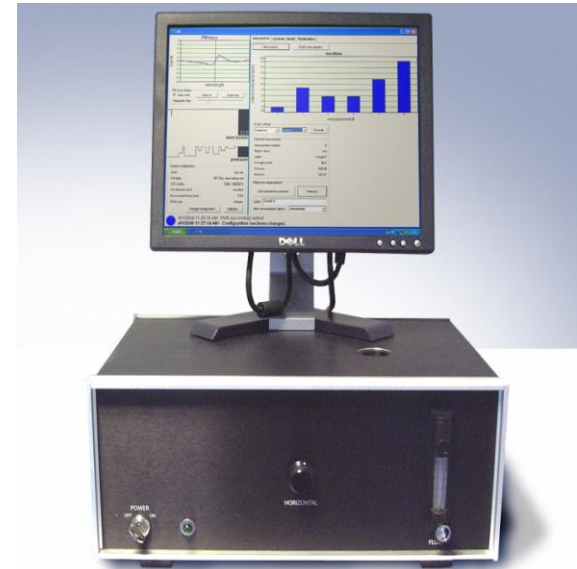
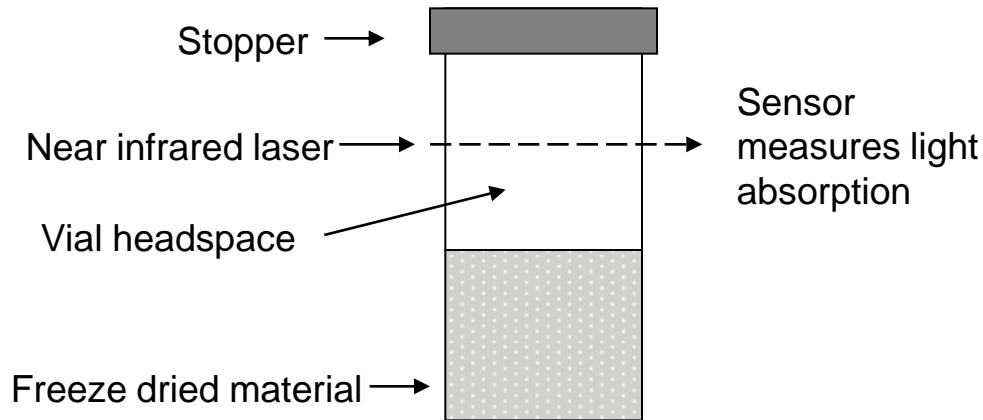
Frequency Modulated Spectroscopy (FMS)

Enables the measurement of water and pressure within the headspace of the vial

- The laser light is tuned to match the internal water absorption frequency at 1400nm
- The amount of laser light absorbed is proportional to the water vapour concentration

Analysis time ~ 5 seconds per vial

- Non-destructive (monitoring same vial over time)
- 100% inspection

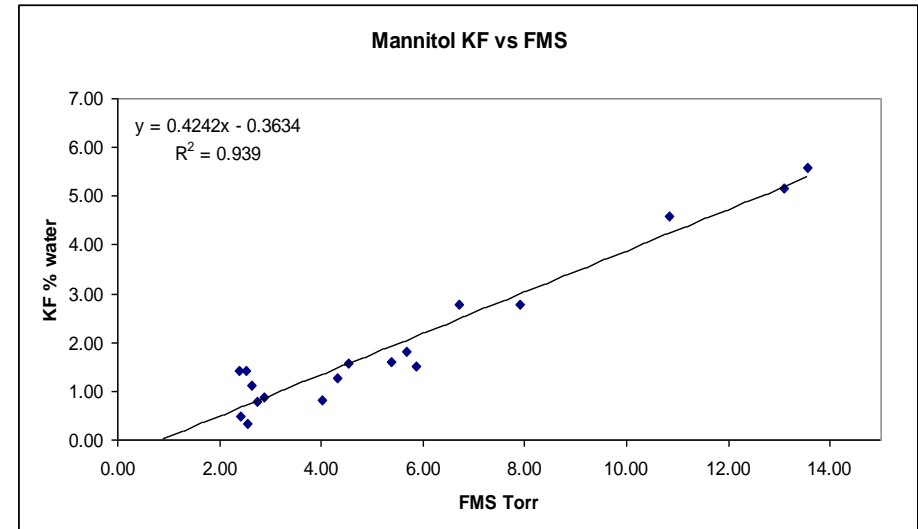
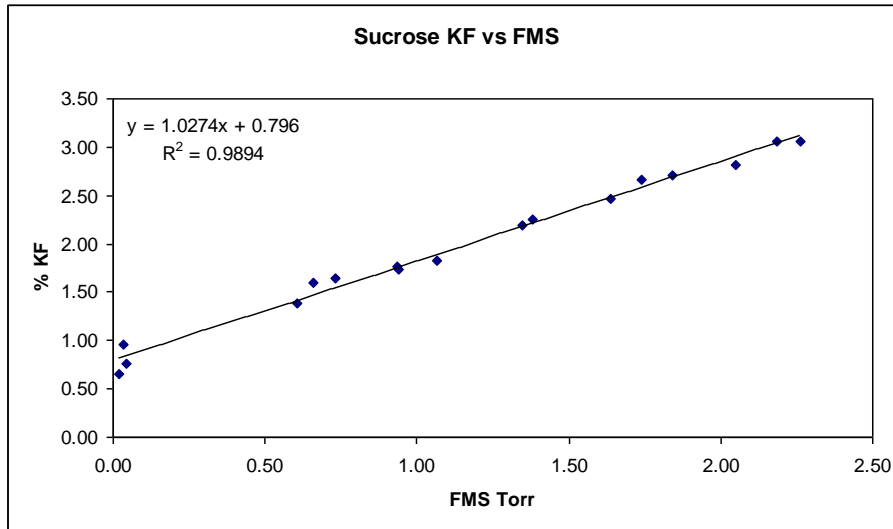


FMS-1400 (Lighthouse Instruments)

FMS and Karl Fischer moisture correlations

Water may be present in a variety of “forms”/locations – free, adsorbed, chemically bound, hydration shells (e.g. of proteins), water of crystallisation

Water can have different association/affinity within the freeze dried material which varies with the formulation



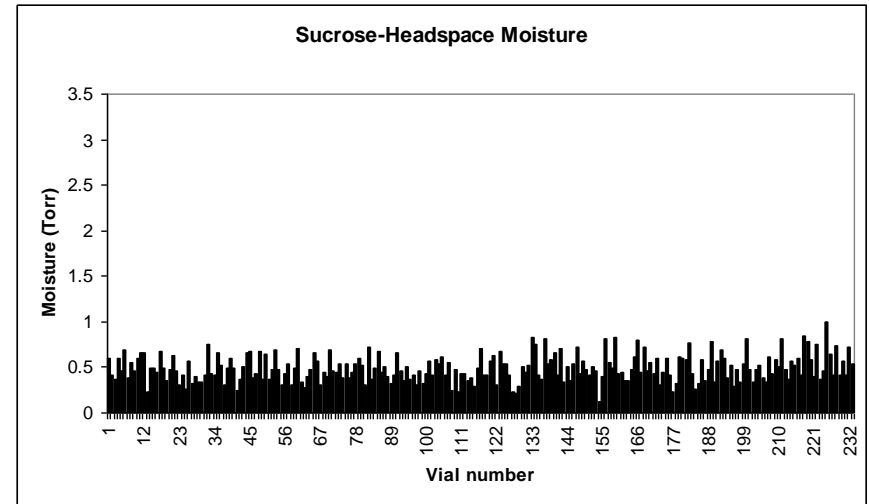
Intercept and gradient vary with the formulation based on intrinsic properties of excipients and active

Moisture mapping for 4% Sucrose

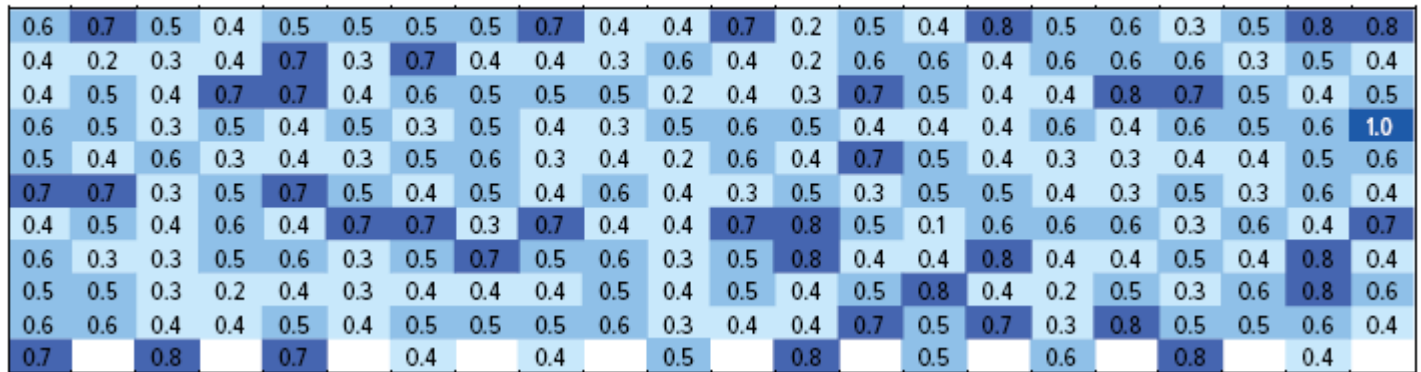
- Primary drying conducted at -40 C, Vacuum set at 50 mtorr

Direct shelf contact

- Uniform headspace moisture
- Lowest 0.2 torr / 1% water KF
- Highest 1.0 torr / 1.8% water KF
- Average 0.49 torr / 1.3% KF
- Standard deviation 0.15



Colour key 0.1-0.4 0.5-0.6 0.7-1.0



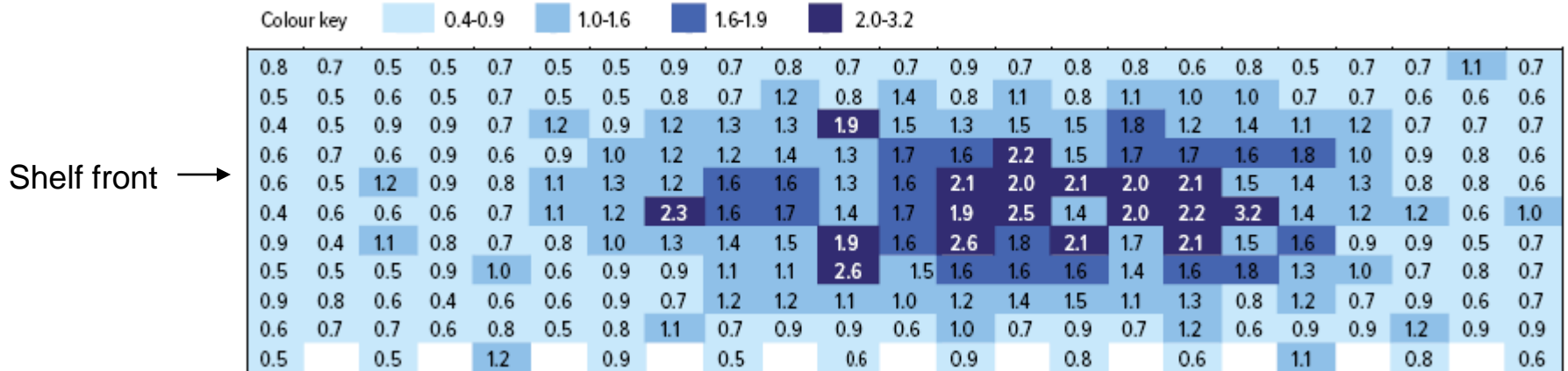
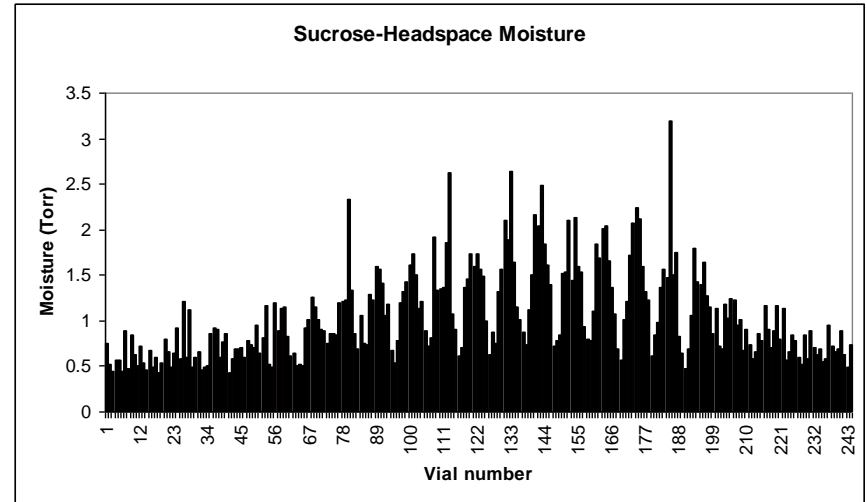
Shelf front →

Moisture mapping for 4% Sucrose

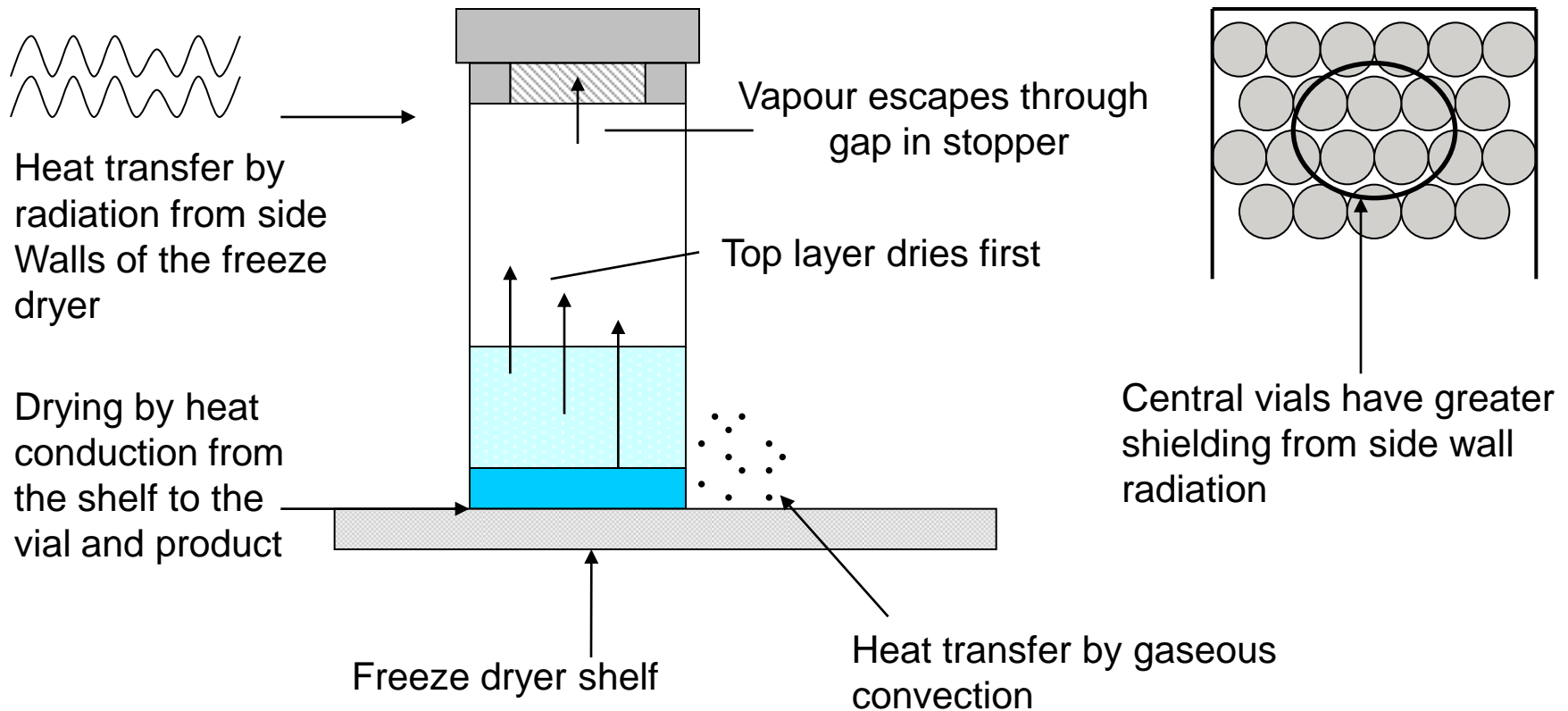
• Primary drying conducted at -40 C, Vacuum set at 50 mtorr

No direct shelf contact (tray)

- High variation in headspace moisture
- Lowest 0.4 torr / 1.2% water KF
- Highest 3.2 torr / 4.0% water KF
- Average 1.05 torr / 1.9% KF
- Standard deviation 0.49



Vial heat transfer in a freeze dryer



Factors affecting moisture variation

Heat transfer by

- **Conduction**
- **Gaseous convection**

Degree of shelf contact

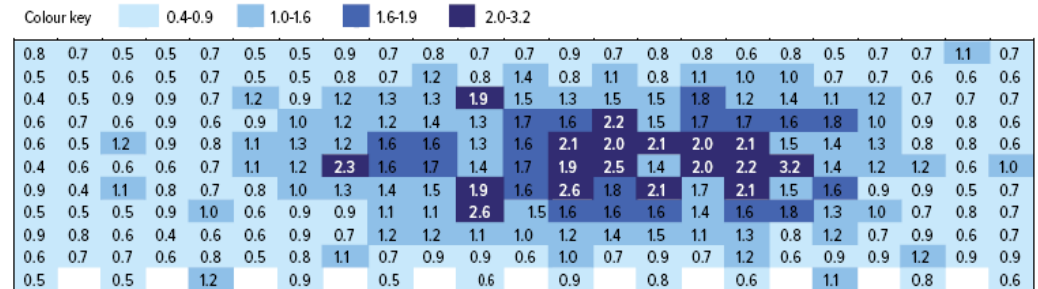
- **Tray**
- **Direct shelf contact**
- **Sample container**

Radiative heating from freeze dryer

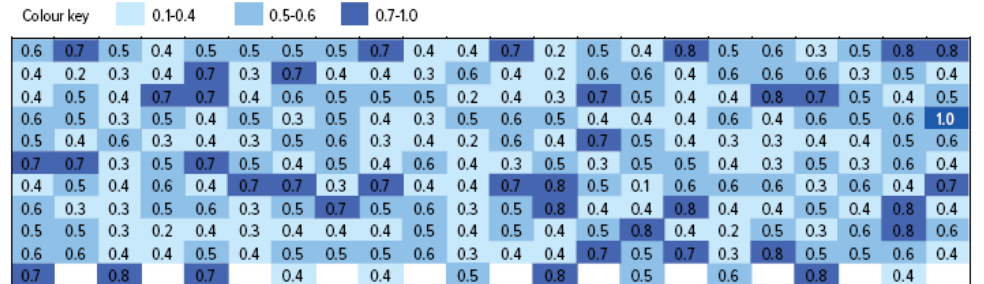
- **Freeze dryer door**
- **Freeze dryer walls**
- **Extent of shielding**

Cycle/processing conditions responsible for observed differences

Samples in a tray

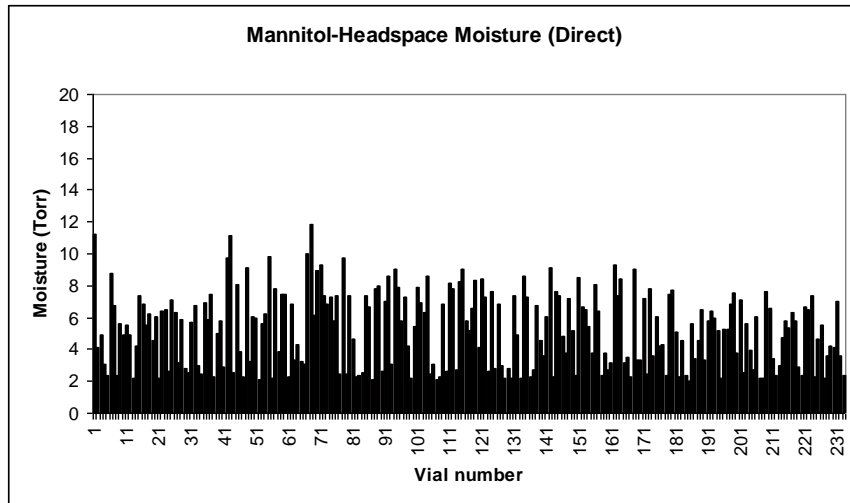


Direct shelf contact

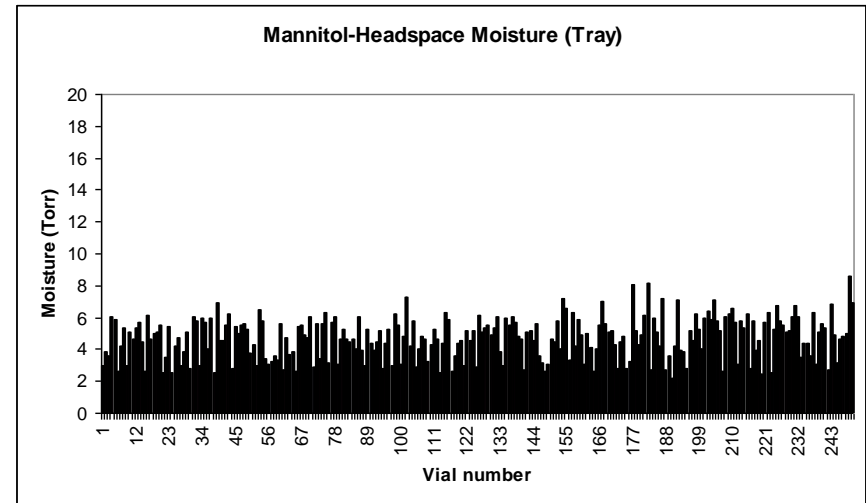


Moisture mapping variations for 2% Mannitol

- Freeze drying cycle involved an annealing step to encourage crystallisation
- Primary drying conducted at -5 C, Vacuum set at 1000 mtorr



- Direct shelf contact
- Average torr 5.24 / ~ 2 % KF
- Standard deviation 2.31



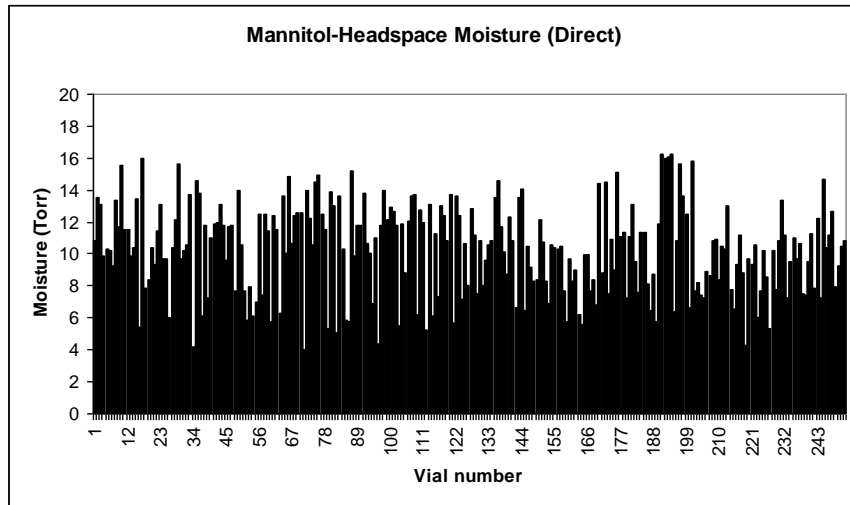
- No direct shelf contact (Tray)
- Average torr 4.72 / ~ 2 % KF
- Std deviation 1.30

Sample sets have a similar moisture content – gaseous convection plays a role!
Significant variation within each sample set

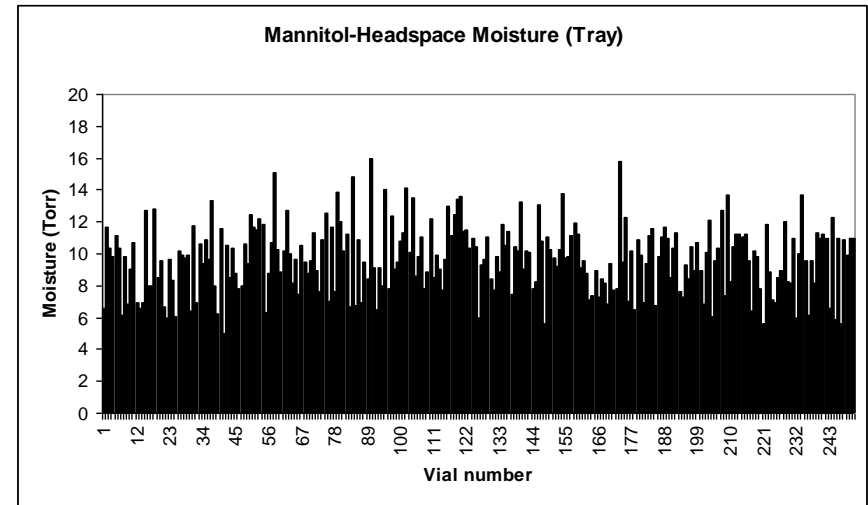
Tray samples have a lower standard deviation – Related to slower cooling rate

Moisture mapping variations for 2% Mannitol

- Freeze drying cycle involved an annealing step to encourage crystallisation
- Primary drying at -40 C, Vacuum set at 50 mtorr, shortened secondary drying



- Direct shelf contact
- Average torr 10.31
- Standard deviation 2.79



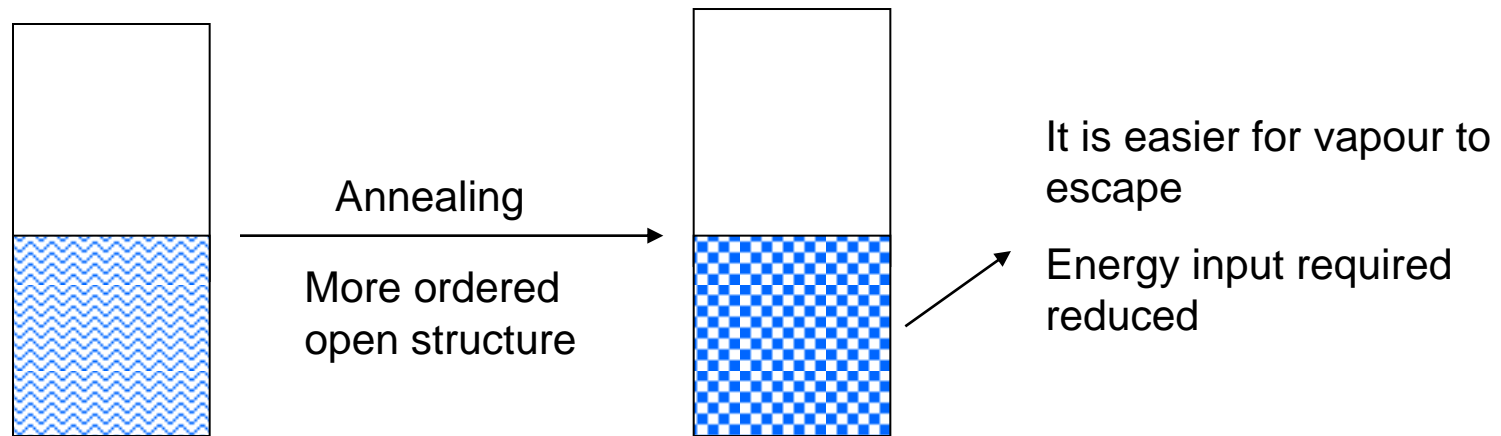
- No direct shelf contact (Tray)
- Average torr 9.69
- Standard deviation 2.12

Sample sets have a similar moisture content – gaseous convection not a factor!
Higher moisture results for both sets due to shortened secondary drying step
Significant variation within each tray

Mannitol – Frozen structure

Annealing – involves cooling and re-warming of the frozen structure

- Encourages crystallisation
- Encourages the growth of larger ice crystals (slower cooling → larger ice crystals)



Structure reduces the impact of heat transfer variation due to shelf contact

Gaseous convection not observed as open structure allows for efficient drying

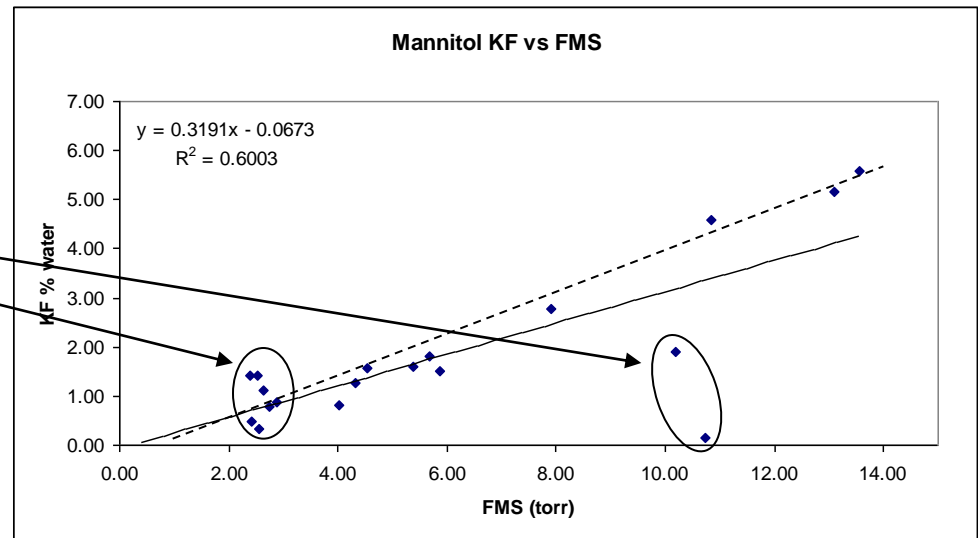
Material structure and treatment can have large impact on the observed moisture

Mannitol – further investigations

Freeze dried mannitol can exist in several forms

- Amorphous mannitol
- Crystalline hydrate(s) of mannitol
- Anhydrous crystalline mannitol - Alpha (α), beta (β) and delta (δ)

Further analysis and closer inspection of the KF/FMS correlation revealed a deviation and lack of correlation for some samples



This variation appeared to be related to a change in the mannitol form (observed by comparing FMS data over several days)

Mannitol – FMS ratio variations

Direct shelf contact

- Higher ratio change
- Random spread (highest ratio change towards front half of tray)

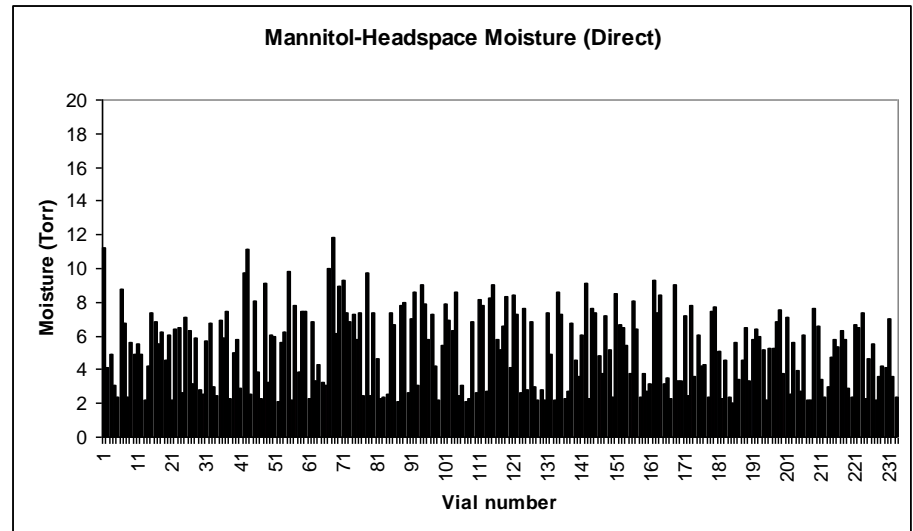
7.8	3.1	3.9	1.8	7.4	4.3	2.3	3.0	3.0	5.1	1.2	3.2	3.8	1.4	1.9	1.2	6.3	1.3	2.7	4.5	4.8	1.7	2.7
2.5	1.2	4.8	1.3	1.3	6.5	1.3	4.5	3.6	3.1	1.5	2.5	1.5	4.9	5.1	1.8	1.7	4.5	4.8	4.8	4.4	1.3	5.0
3.0	2.2	1.3	4.0	4.8	1.1	1.5	1.1	1.0	4.5	1.1	3.4	1.1	1.9	2.5	1.3	1.3	5.0	1.8	2.2	2.0	4.8	2.1
1.7	5.7	5.0	3.1	1.9	5.3	5.9	4.7	4.1	1.8	1.1	4.1	1.2	1.5	1.0	1.1	4.9	2.2	4.2	5.0	1.3	4.4	1.4
1.2	4.7	3.2	4.9	1.1	1.6	4.9	0.9	4.8	1.0	3.1	1.5	1.1	2.6	5.4	3.1	0.8	1.1	3.9	1.3	1.6	5.2	2.5
6.6	2.4	1.4	1.1	5.6	3.6	1.0	4.6	P	2.4	1.2	5.0	4.8	5.0	2.4	1.3	P	1.9	3.8	4.1	2.9	1.3	1.2
4.1	3.4	2.9	4.3	1.4	4.7	5.0	2.2	1.2	3.5	3.1	3.9	2.5	1.1	3.8	4.9	4.1	1.2	3.7	2.1	3.1	2.4	4.5
1.3	2.2	1.3	3.0	2.6	1.1	5.8	1.1	3.2	2.9	4.2	1.2	1.1	2.8	2.0	1.3	1.6	1.1	1.2	1.4	3.3	4.1	2.0
4.3	4.3	1.2	1.4	4.3	4.7	4.9	1.1	5.5	3.1	1.1	3.2	4.8	3.8	1.5	1.2	4.4	3.0	3.9	3.6	4.4	1.2	4.3
1.1	1.2	4.1	6.1	1.1	1.8	4.8	1.2	1.5	3.5	4.3	1.3	3.3	2.4	4.4	1.1	2.0	1.9	4.4	1.2	3.6	2.0	4.7
2.8		3.5		2.5		4.8		5.5		5.7		1.0		4.5		1.9		1.2		2.1		

Ratio change= Day 3 / Day 1 1-1.4 1.5-3 3-5.5 5.6-8 Green = no / small change

High level of variation observed for headspace moisture

Standard deviation 2.31

Indicate headspace moisture variation within a shelf related to the different proportion of mannitol crystalline forms



Mannitol – FMS ratio variations

No direct contact (tray)

- Lower ratio change
- Random spread
(Higher ratio changes towards tray edge)

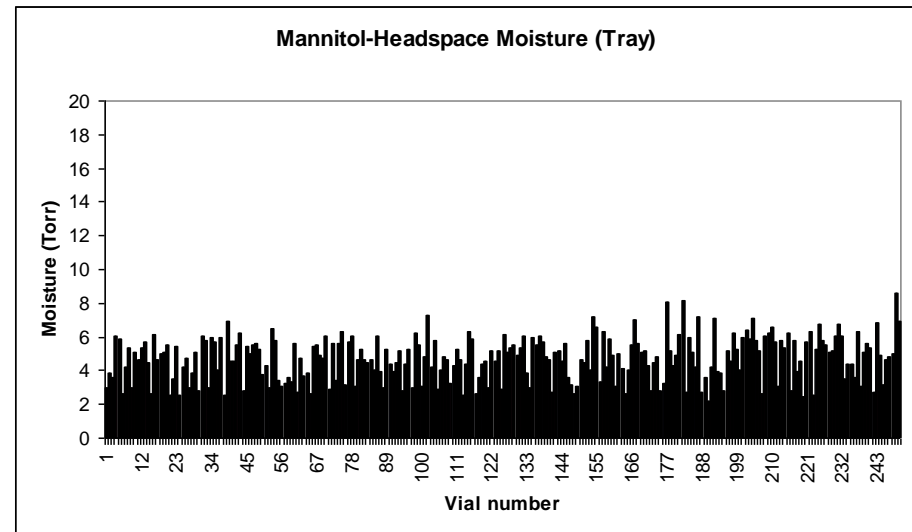
1.5	2.9	1.8	1.5	3.7	3.8	2.0	2.8	1.9	1.9	1.3	3.3	2.9	3.2	1.6	2.2	3.4	2.2	1.2	2.6	3.4	1.2	3.0	2.4
1.9	3.4	3.4	3.1	1.3	1.4	1.2	3.2	1.5	3.3	1.9	1.1	2.1	2.8	1.2	3.4	2.8	1.0	1.6	1.5	2.2	2.2	3.6	1.3
1.8	2.2	1.2	2.9	1.7	1.2	2.6	1.1	3.0	1.1	2.1	1.1	2.9	1.3	1.2	3.0	1.6	3.8	2.4	2.5	2.2	3.0	2.1	2.8
3.4	1.2	3.1	1.4	1.6	1.1	1.0	2.3	1.3	3.2	1.6	1.3	3.6	1.9	1.4	1.1	1.4	2.4	1.9	1.7	1.4	1.1	1.8	2.9
3.1	3.1	1.9	2.3	1.2	1.4	1.4	3.0	1.2	2.8	1.2	2.7	3.1	1.1	3.1	2.9	1.1	2.8	2.2	1.9	2.2	2.2	2.8	1.4
1.3	3.0	1.2	1.1	2.8	1.2	P	1.1	2.9	1.1	2.7	1.2	2.1	3.0	2.6	1.2	P	1.1	1.7	2.9	1.9	1.9	3.2	2.8
2.9	3.2	1.8	3.7	2.7	2.7	3.0	1.8	2.8	2.9	2.9	3.0	2.8	2.8	1.7	1.0	1.6	3.1	3.2	3.1	2.3	2.3	1.5	2.3
2.8	3.2	2.5	2.7	1.5	1.0	2.6	2.1	1.4	3.1	2.1	2.7	1.2	3.1	3.2	1.4	3.0	2.7	1.0	3.0	1.1	2.1	2.7	3.2
1.2	3.2	1.2	2.9	1.7	2.9	1.0	2.1	3.2	1.8	1.1	2.5	1.2	2.1	2.0	3.1	1.2	2.1	3.3	1.2	2.7	2.2	1.4	4.6
3.7	1.2	3.6	3.4	1.1	1.4	3.4	2.9	3.1	3.1	2.9	1.2	2.3	1.5	1.4	3.0	1.4	4.0	2.1	3.1	1.8	3.6	2.5	3.5
2.4		3.0		3.0		1.6		1.0		2.1		2.1		3.3		3.6		3.6		2.2		3.2	

Ratio change= Day 3 / Day 1 1-1.4 1.5-3 3-5.5 5.6-8 Green = no / small change

Lower level of variation observed for headspace moisture

Standard deviation 1.30

Indicate slower heat transfer results in more controlled crystallisation and a smaller variation in mannitol form



Summary

Headspace moisture analysis can be used as a non-destructive method to further our understanding of the factors involved in obtaining a uniform moisture content

Processing factors

- Efficiency of heat transfer – conduction and convection, container material
- Degree of shelf contact e.g. tray/no tray, container shape
- Radiative heating – larger shelves = fewer vials exposed to side wall radiation
- Annealed or non-annealed - ice crystal size, pathways for vapour to escape
- Cooling and re-warming rates

Excipients/active material

- Material type/structure e.g. amorphous or crystalline, material complexity

Important to fully understand reasons for moisture variation due to processing and material choices.

This understanding can assist in validation and in assessing any changes made

Thank you

Any questions?

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