

An impedance-based process analytical technology for monitoring the lyophilization process

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Abstract

The aim of this work was to develop a minimally-invasive, impedance spectroscopy method as a novel process analytical technology for monitoring the freeze drying process. This involved the application of planar electrodes, mounted externally to a conventional glass freeze-drying vial, coupled to a high-impedance analyser. The pseudo-relaxation process arising from the composite impedance of the glass wall and product interface was recorded over a frequency range 10^1 to 10^6 Hz for a surrogate formulation comprising 2.5% sucrose. Features of the process (i.e. the peak amplitude, C''_{peak} and characteristic peak frequency, f_{peak}) were monitored along with the product temperature data during the entire cycle. It was demonstrated that f_{peak} is strongly coupled to the temperature of the product (through the dependence of the product temperature on the electrical resistance of the product) whereas C''_{peak} is dependent on the extent of ice sublimation and hence can be used to measure the rate of drying and end point of primary drying. This feature provides a distinct advantage over thermocouple measurements which are restricted to end point detection only. The potential to predict the end point of a cycle from C''_{peak} vs time profiles is highlighted in this work.

Key words Freeze drying, impedance spectroscopy, process analytical technology for freeze drying, end of primary drying

1 Introduction

Lyophilisation is used for the preservation of thermo labile products in the biotechnology, pharmaceutical and food industries. The benefits of the process include extended shelf life and more economic storage and transportation relative to the liquid form of the product (*Pikal, 2002*). However, due to high development and manufacturing costs this process is invariably reserved for ‘high value’ products.

A quality by design (QbD) approach might significantly reduce both the product development time/cost and subsequent operational costs through a more rational design of the product and an optimised (i.e. shorter) freeze-drying process. The QbD philosophy provides a structured and systematic approach to the study of the various dependencies between formulation and process parameters that influence critical quality attributes of product. In brief, QbD requires the development of a control space (which is partly informed by the application of process analytical technology: PAT) to ensure that the process will stay within predetermined operational limits (once in routine production/manufacture) and thereby deliver a product that meets its target quality product profile. This is not to say that all process analytical technologies will be used in the routine manufacture of the product. Those technologies, providing data on the batch as a whole, may indeed be useful in production. However, many technologies (especially those intended for single vial measurements) can be used to effect within the product development stage and process scale-up.

A variety of techniques are employed to estimate the heat and mass transfer rates in the closed system of the drying chamber and condenser, viz. gravimetric measurements (*Xiang et al., 2004*), product temperature sensors and heat transducers (*Chen et al., 2008*), manometric temperature measurements (*Tang et al., 2006*), tuneable diode laser absorption spectroscopy (*Kuu et al., 2010*), and visual monitoring of the ice interface (*Chen et al., 2008*). These techniques have been reviewed elsewhere (*Patel and Pikal, 2009*). PAT for freeze-drying process monitoring may be sub divided in those which are, in essence, intended for measurement of individual vials and those which measure ‘across’ the whole batch. Here we briefly examine those individual vial techniques which are most

relevant to the development of a new 'single vial' PAT technology, viz. the gravimetric approach and those methods based on the use of temperature sensors. Temperature sensors may be used to calibrate any new PAT system that might otherwise provide a surrogate method for temperature measurement, whereas the combination of a temperature sensor with the gravimetric approach may provide a suitable means for evaluating any new PAT system in terms of its potential for the detection of the primary drying end point.

The gravimetric method, such as the purposefully designed microbalance has been shown to measure mass transfer rates at different freeze drying conditions, thereby helping assess the impact of chamber pressure and shelf temperatures on the rate and duration of primary drying (Pikal et al., 1983; Xiang et al., 2004). However, the cake structures of those samples dried in the vials attached to the microbalance may differ from their conventional counterparts due to the inevitable changes to the heat transfer rate that is associated with frequent lifting of the vial from the shelf (Xiang et al., 2004). Moreover, the vial connected to the holding arm of the microbalance may not be positioned within the usual hexagonal arrangement when multiple vials are loaded into the freeze-drier and therefore the thermal response from the microbalance vial will be inevitably non-representative of a full shelf scenario.

Temperature sensors, particularly the resistance temperature detectors (RTDs) and thermocouples, which are frequently used for the monitoring of product temperatures over the entire process, are considered to be non-representative of the entire batch owing to un-representative freezing rates and altered sublimation kinetics, conferred by the invasive nature and thermal contributions of these devices (Patel et al., 2010; Rambhatla and Pikal, 2003). Moreover, the multi-dimensional drying characteristics of ice from within the vial often leads to inaccuracies in the measurement of sublimation endpoints, especially if the probe is positioned near the glass wall (Song et al., 2005) One corrective measure to accommodate the resultant differences in drying rates between those vials loaded with the thermocouples and those without is to add a soak period equal to 10-30% of the target sublimation time, before switching to secondary drying stage (Rambhatla and Pikal, 2003; Tang and Pikal, 2004).

Heat transfer rates have also been measured using heat flux transducers (essentially a pair of thermocouples) which take into account the temperature difference between the surfaces of exposure; i.e. the outer surface of shelf and lower surface of vial bottom (Chen et al., 2008). This necessitates the positioning of a pair of planar thermocouples in between the top surface of the shelf and the external (base) of the vial. Although this has the advantage that no sensor is inserted into the product itself, the obvious drawback is that the thermal mass of the sensors may alter the heat flux through from the shelf through the base of the vial.

The increase in electrical resistivity (often measured using an alternating current frequency of 1 kHz) that occurs with a liquid to solid phase transitions (Rey, 1960) has been used in combination with the conventional formulation development tools (differential thermal analysis and freeze drying microscopy) to identify critical process events (ice solidification and eutectic formation) against which process set points can be defined (i.e. the product temperature during the pre-freezing stage) (Hajare. et al., 2012; Luis Rey and May, 2004; Oetjen, 2007). However, many of these systems are not configured for in-situ measurement within a freeze-dryer. Those which do measure the solution resistance during the freeze-drying process employ a pair of pin-electrodes which are immersed directly into the solution within the freeze-drying vial (e.g. the CHRIST LyoRx measurement system)

This article presents a new type of individual vial monitoring system, based on impedance analysis, which has shown some potential for characterizing lyophile attributes and process end-points (Smith et al., 2011). The principal difference between this new approach and other individual vial technologies is that the sensor is neither introduced into the vial nor placed beneath which could otherwise impact the freezing/crystallization behaviour or the rate of primary drying. The current system is multi-channel and so permits the sequential measurement of a number of individual vials. Hence a number of zones across a single shelf may be measured in one experiment, to provide some indication of hot/cold spots across the shelf. The measuring vials can be placed at any position within the usual hexagonal array (that maximises the load on the tray in the drier) thereby enabling a degree of thermal mapping of the shelf. The measurement system is minimally invasive to the product which

in turn reduces the thermal impact on the process. This article provides a brief insight on the applications of this technology in the characterization of the lyophilization process, using a surrogate formulation containing 2.5% w/v sucrose in deionised water. The main focus of this article is the determination of the end points of (i) the freezing process and (ii) the primary drying stage.

2 Experimental

2.1 Materials

Sucrose from Sigma Aldrich was used as received from the supplier. Single distilled water, from ‘all-glass’ apparatus, was used throughout the study.

2.2 Equipment

A bespoke multichannel high precision impedance analyser was designed and manufactured by the authors comprising several (5 in this embodiment) specially designed current to voltage (IVC) converters (Fig. 1), a data acquisition system (including the calibration routine and the measuring and primary data treatment software, Fig. 2), with a useable bandwidth of 10 Hz to 1 MHz (Fig. 3). The measurement installation also comprises a number of special measurement vials (Fig. 4) connected through miniature coaxial cables to a junction box (located within the freeze-drier) and a vacuum pass through from the freeze-drying chamber to the external environment (where the IVC is located).

The IVC design (Fig. 1) is extremely simple, compact and inexpensive to manufacture, thereby reducing the constraints on the number of channels that could be built into the system

However this simplicity leads to a rather sophisticated calibration based on expression (1) that is obtained as a solution of system of equations mathematically describing the IVC operation.

$$W_{CL}^* = \frac{V_1^*}{V_0^*} = \frac{W_{OL}^* Z_{FB}^* Z_P^*}{Z_{FB}^* Z_P^* + Z_{IN}^* Z_P^* + Z_{IN}^* Z_{FB}^* - W_{OL}^* Z_{IN}^* Z_P^*} \quad (1)$$

where W^*_{CL} is a closed loop complex transfer function, W^*_{OL} is an open loop transfer function of the operational amplifier, and Z^*_{IN} , Z^*_{FB} and Z^*_P are complex impedances of a reference object (for that purpose we use a 1 pF capacitor with air dielectric), feedback circuitry and parasitic input impedance, respectively, of the operational amplifier and connecting cable respectively (asterisk in superscripts denotes complex values). During the calibration routine (Fig. 2, LHS), the complex spectra of W^*_{OL} and the complex spectra of Z^*_{FB} are recorded during the calibration routine, for each channel and stored in a calibration file. Separately, the value of parasitic impedance is measured for each channel and again stored in the calibration.

Then by resolving equation (1) with respect to Z^*_{IN} the working formulae (2) is obtained, which is used subsequently to calculate the complex impedance of an object under test (in our case the measurement vial and its contents).

$$Z^*_{IN} = ((W^*_{OL}Z^*_{FB}Z^*_P) / (V^*_1/V^*_0) - Z^*_{FB}Z^*_P) / (Z^*_{FB} + Z^*_P - W^*_{OL}Z^*_P) \quad (2)$$

Though the calibration routine is rather complex and time consuming, once been done it yields exceptional accuracy, sensitivity and resolution of the IVC, as listed here: Measurement range up to $10^{14} \Omega$ (impedance magnitude) with a phase angle resolution of $\sim 0.001^\circ$ or, in terms of minimal measurable capacitance, the designed IVC has a resolution of $10^{-16}F$ in frequency range from 10 Hz to 1MHz (Fig. 3). This measurement resolution is sufficient to measure changes in the capacitance of the test system, which typically range from 0.6 pF (for the liquid filled vial at the start of the process) to 0.1 pF (for the dried product, at the end of the process), across the frequency range of 10 Hz to 1 MHz with a precision of 0.1%.

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The measurement vial (Fig. 4) is a standard 10 ml, clear glass, tubing vial (Schott) with an added electrode system, which consists of an identical set of stimulating/sensing electrodes (18 x 5 mm), each with a surrounding grounded guard electrode which prevents electrical current leak between stimulating and sensing electrodes over the outer surface of the vial. The electrode system was manufactured from adhesive copper foil and affixed on opposite sides of the external surface of a vial, just above the bottom curvature in order to avoid electrical contact (and hence grounding) with the shelf.

The decision to locate the electrode system on the external surface of the vial was based on a number of considerations: (i) The low thermal mass of a foil electrode, compared with a pin electrode of sufficient mechanical strength to prevent its flexing, reduces the heat flow to/from the sample, (ii) Having the electrodes external to the vial would not contaminate the sample, while reducing the impact on the crystallization dynamics by not introducing further nucleation sites from the presence of a foreign body within the liquid, (iii) The impedance measured by external electrodes will be significantly higher than that measured by internal electrodes; It therefore follows that the influence of the cables impedance on the overall measurement will be insignificant, thereby reducing the potential for artefacts being introduced, e.g. from the effect of spatial temperature gradients in relation to the positioning of the cables, (iv) an electrode system placed internal to the vial, which comprises two straight pins necessarily requires a rather bulky adapter (to maintain the parallel geometry of the pins) which can only be located over the neck of the vial, and therefore inevitably impedes the efflux of vapours during primary drying; It is therefore difficult to imagine that such a system may be used in the primary drying stage without significantly impacting the drying rate. The bulky connectors would also preclude the deployment of an automatic vial stoppering mechanism. The last but by far the most important consideration is that the external electrodes do not give rise to a fractal electrode polarisation, which is inevitable when metal electrodes are in direct contact with a sample. This makes the interpretation of spectral data much simpler and straightforward; and as we shall demonstrate in future articles this feature enables the extraction of actual values for both the sample resistance and sample capacitance.

The spectral information from the analyser is transmitted to the measurement control software, which in turn displays the measured response in graphical as well as text file formats. The control software also permits the user to define the impedance scanning protocol in terms of (i) the frequency range (the band width of the system is 10 Hz to 1 MHz, but the useful frequency range is typically 100 Hz to 250 kHz), (ii) the stimulating voltage (typically 2V), (iii) the scanning interval, i.e. the time between the measurement sequence on all 5 test vials (typically 5 min), (iv) the number of integration cycles per spectrum (typically $n = 4$), and (v) the length of the cycle (typically 40-50 h).

A HETO 08 Freeze drier; with installed the measurement system, was used for the investigation of the drying process.

2.3 Methods

To ensure the efficacy of the guard electrode it is essential that the outside of the vial, especially the area of glass in between the stimulating electrode and guard electrode, is scrupulously clean. To this end, a routine cleaning process was implemented which involved wiping over the surface with a methanol soaked cotton swab and then wiping over with a dry swab.

A 2.5% w/v sucrose solution was prepared in distilled water. Aliquots (3.0 ml) were introduced to the measurement vials using a 5 ml syringe and a micro filter (0.1 μm pore size) to ensure particulate free transfer of the liquid. The measurement vials were arranged in a straight line (position 1-5 from Left to-Right) behind the Perspex door to facilitate photographic imaging (Fig. 5). The vial at position 5 also contained a type-K thermocouple in order to assess what might be a representative product temperature. The lyophilisation cycle was initiated after the connections between the measurement vials and the impedance interface were checked by performing a single test scan using the control software.

The freeze-drying protocol is described in Table 1. Impedance measurements were recorded over the entire lyophilisation cycle. The duration of each frequency sweep (10 Hz – 1 MHz) per test vial, was 45 s, resulting in a scan time of 3 min 45 s for the set of 5 test vials. The total interval from the start of

one measurement sequence (i.e. a set of sequential measurements on the series of 5 test vials) to another was set at 5 min.

Freezing related phase changes were also documented using a Canon D450 SLR camera in time lapse photographic mode. Images were recorded at an interval of 2 min and the onset time for ice formation documented for each vial. Special note was made of the onset time of the thermocouple containing vial in relation to the others.

3 Results and discussion

Changes in phase (e.g. ice formation), the completion of freezing, and the end-points of primary and secondary drying are all detected by this method through changes in the composite impedance of the formulation-vial-electrode assembly. Fig. 6 a/b show the real and imaginary components of the impedance spectra from a typical cycle. Over the entire response surface one can see that the imaginary impedance is between 10 to 100 times greater than the real part impedance, a fact which reflects the capacitive (rather than conductive) nature the composite impedance of the formulation-vial-electrode assembly. Although the impedance is dominated by the sample capacitance, one can nevertheless observe the resistance of the composite impedance from the low frequency asymptote on the real part spectrum (see arrow on Fig. 6A) during the initial phase of the cycle. This plot provides the first indication that the impedance of the test object (i.e. the formulation/vial/electrode assembly) has a direct correlation with the temperature and physical state of the formulation within the vial.

However, the transitions of phase (from liquid to ice) and the impact of temperature on the response surface are more easily determined if the complex impedance ($Z(\omega)$)

$$Z(\omega) = Z'(\omega) + i Z''(\omega) \quad (3)$$

is displayed as a complex capacitance ($C(\omega)$)

$$C(\omega) = C'(\omega) + i C''(\omega) \quad (4)$$

where

$$C(\omega) = 1/i \omega Z(\omega) \quad (5)$$

The formulae deriving real and imaginary capacitances are given in eq. 6 and 7 respectively

$$C'(\omega) = -Z''(\omega) / \omega(Z'(\omega)^2 + Z''(\omega)^2) \quad (6)$$

$$C''(\omega) = -Z'(\omega) / \omega(Z(\omega)^2 + Z'(\omega)^2) \quad (7)$$

Objects that behave like capacitors have a negative imaginary impedance, and therefore by definition the imaginary capacitance of these objects is also negative.

Fig. 7a and b show the response surfaces for the real and imaginary capacitance. In the frozen state (up to 9.5 h, and prior to starting the primary drying phase) the response surface is characterised by a step-like transition in the real part capacitance and a peak in the imaginary part capacitance, as the frequency is increased from 100 Hz to 250 kHz. This response resembles that of a classic relaxation process within a dielectric material. However, in this case the observed behaviour is instead an inevitable consequence of the fact that the object under test is in effect a composite impedance of a number of discrete elements, i.e. the conductive product within the glass vial and the capacitive glass walls of the freeze-drying vial. It is the series disposition of these two elements which leads to the observed phenomena: a interfacial polarization whereby the glass wall is charged through the resistance of the sample. The temperature dependency of the process gives rise to the observed frequency dependency, i.e. the apparent relaxation process (or pseudo-relaxation) in the frequency domain spectrum.

Although there is not an exact physical description of the measured impedance, the equivalent circuit model (Fig. 8) satisfactorily defines the physical origin of the pseudo-relaxation process.

It is the characteristics of the pseudo-relaxation process that is used to ‘follow’ the progression of the freeze-drying cycle during the early stages of the cycle. Of the real and imaginary capacitances, it was the imaginary capacitance response surface that was used to demonstrate changes to the physical properties of the system. Rotation of the response surface of imaginary capacitance spectra as a function of time (shown first in Fig. 7b) through 90 degrees (Fig. 9) highlights the observation that the pseudo-relaxation peak has a pronounced dependency on physical state of the solution, and undergoes distinct shifts in peak frequency (f_{peak}) and peak amplitude (C''_{peak}) during each stage of the process.

These characteristic changes in the pseudo-relaxation peak are more clearly demonstrated by overlaying the imaginary capacitance spectra from individual time points associated with each stage of the process. We shall demonstrate this for the freezing, annealing and primary drying stages. However, once primary drying is complete, the pseudo-relaxation peak has diminished to zero and therefore may not be used to follow the progress of the cycle. At that point, however, one can still see changes in the impedance response of the sample through changes in the value of C' or C'' at some representative frequency (e.g. 1 kHz). Given the low mass (and hence dielectric constant of the product) then the lower limit of capacitance is in effect that of the empty vial.

3.1 Impedance Spectrum Profiles

3.1.1 Spectrum profiles during cooling (phase I)

Product cooling (from 17 to -7 °C) resulted in a small decrease in the pseudo-relaxation frequency (f_{peak}) of ~6% of the initial value (where the initial value is 90 kHz at 18 °C) and an even smaller decrease in the peak amplitude (C''_{peak}) of ~2% of the initial value (0.93 pF at 17 °C).

The decrease in the relaxation frequency is consistent with what one might predict from an approximation for the time constant of the process ($\tau = 1/2\pi RC$). As stated previously, R is the resistance of the material within the freeze-drying vial and C is an approximation for the CPE element. The approximation works owing to the fact that the phase angle for the interfacial impedance (represented by the constant phase element, CPE) is close to 90 (e.g. 89.2° when the sample is in the liquid state). Given that the resistance of an aqueous solution (in the liquid state) has negative temperature dependence and the capacitance of a solid (such as glass) also has negative temperature dependence (albeit much smaller than that for the resistance of the liquid) then the overall result is that the relaxation time increases and the corresponding relaxation frequency decreases with a decrease in temperature. The specific dependence of the pseudo-relaxation peak on the product temperature, during cooling, is explained further following the analysis of spectral peak data using peak finding software.

3.1.2 Spectrum profiles during freezing (phase I to II)

The phase transition from liquid to solid state is demonstrated in changes to both the peak frequency (f_{peak}) and peak amplitude (C''_{peak}) of the pseudo relaxation process: In contrast to the relatively small changes that are manifest on cooling through ~ 20 °C (as reported above), the pseudo relaxation peak undergoes a more dramatic transition as the product solidifies: The peak frequency (f_{peak}) shifts to the lower frequencies by a factor of 30-100 (i.e. approximately 1.5-2 decades) while the peak amplitude (C''_{peak}) increased by a factor of 0.3, i.e. 30% (Fig. 11).

The shift in relaxation frequency by almost 2 decades is consistent with the fact that the solution resistance increases by almost 100 fold on freezing, as liquid water is converted to ice (Chin et al., 2007). The increase in the peak amplitude (C''_{peak}) may be rationalised if we consider the dielectric constant of the interfacial region between the glass wall and the sample. Given that the dielectric constant of ice ($\epsilon \sim 100$) is greater than that of liquid water (by a factor of $\sim 25\%$) then the increase in peak height of 30% is consistent with the high dielectric constant of a frozen aqueous solution relative to its liquid counter-part.

3.1.3 Spectrum profiles during Annealing (Phase II to III)

During annealing, a 13 °C increase in the product temperature resulted in $\sim 13\%$ increase in the logarithm of the pseudo relaxation peak frequency ($\text{Log } f_{\text{peak}}$) (Fig 12). These results suggest a greater sensitivity to temperature changes when the solution is in the frozen state compared to the liquid counterpart (in which $\log f_{\text{peak}}$ decreases by 6% when the temperature was decreased by ~ 25 °C). Similar results were reported by Bettelli et al, who reported a strong correlation of relaxation frequency with the temperature of the ice; whereby the relaxation frequency increases with the increase in temperature of the pre-frozen ice (Bittelli et al., 2004). This relationship defines the potential application of impedance measurements in the analysis of the product temperature (especially in the frozen state) without insertion of a thermocouple into the object.

3.1.4 Spectrum profiles during primary drying (III to IV)

The primary drying stage starts by lowering the chamber pressure (to 0.1 mBar) over a period of 15 min. A reduction in the product temperature (~ 5 °C) over this period confirms the onset of primary drying following the application of vacuum. Simultaneously, $\log f_{\text{peak}}$ decreased by 9%, which is consistent with the temperature dependence of peak frequency as observed above (at stage II). The gradient in the $\log f_{\text{peak}}$ under the applied vacuum appears to be twice that of observed on heating the product during the annealing phase.

After, the initial drop in temperature, the amplitude of the pseudo-relaxation peak ($\log C''_{\text{peak}}$) continuously decreases with the progression of the sublimation process. There is a natural consequence of a reduction in the interfacial area (a) between the ice and the glass wall (as the ice front recedes down the inside of the vial) which in effect defines the magnitude of the interfacial capacitance (or more specifically the magnitude of the constant phase element) through the well-known relationship, $C \propto \epsilon \cdot a/d$ (where ϵ and d are the dielectric constant and thickness of the interfacial layer, respectively and a is the area of interfacial layer). In contrast, the peak frequency (like the temperature as measured by the thermocouple) remained relatively stable during the primary drying phase (Fig 14b).

At 23 h (14b) the peak becomes less clear, which has consequences for the determination of the end point of the drying cycle (as explained later). Therefore, in the context of sublimation, the magnitude of the peak i.e. the imaginary capacitance can be termed as an index of the product ice content. However, after ~ 22 h of freeze drying cycle time, the pseudo-relaxation peak becomes rather small, and the peak not so well defined as the low frequency side becomes impacted by measurement uncertainties.

3.2 Peak profiles

Data files acquired by the control software were subjected to data treatment software which uses a peak finding logic to display the data in a time slice of the peak amplitude (C''_{peak}) and/or peak

frequency (f_{peak}) (Fig. 15a and 15b, respectively). Alternatively, one can simply select a time slice of the real and imaginary capacitances at a specific user-defined frequency, e.g. 1 kHz. Using the example of 1 kHz, the displayed parameters would then be termed $C'(1 \text{ kHz})$ and $C''(1 \text{ kHz})$, respectively (Fig. 15c and 15d). The derivatives of any of the output parameters were then estimated separately using Microsoft Excel.

3.2.1 Peak Profiles during cooling (phase I) and freezing

Over the freezing stage (i.e. the first three hours) of the cycle, the measurement system records dramatic changes in the peak frequency and peak amplitude (Fig. 16). Primarily these relate to the phase change from liquid to solid, though it can also be said that the temperature in either state also has a significant impact on the measurement parameters.

The characterization of the freezing process by use of temperature sensing technologies is invariably defined in terms of three discrete but inter-connection stages, i.e. A-B onset of ice formation, B-C ice crystallization, and C complete solidification (Kasper and Friess, 2011; Nakagawa et al., 2007; Rahman et al., 2002).

The onset of ice formation (A-B) was evident from the increase in the temperature of the system, as the energy from the exothermic process fails to dissipate from the vial. This event is mirrored by an instantaneous increase in the log of the pseudo-relaxation frequency (1-2%). This rather small increase in $\text{Log } f_{\text{peak}}$ is in contrast to the two fold increase in the peak amplitude C''_{peak} values (0.097 to 0.178 pF at $\sim 0.6 \text{ h}$) that arise during the main ice crystallization stage (B-C), suggesting that the latter parameter is an effective measure for the phase changes in the sample.

The end point of the solidification phase is considered as the point whereby the rate of cooling increases following a transient ramp in temperature (associated with exothermic process of crystallization). During the equilibration phase (point C onwards) the product temperature approaches the baseline defined by the shelf temperature. Over the same period, the magnitude of $\text{log } f_{\text{peak}}$

decreased accordingly. In contrast, the C''_{peak} values increased during the temperature hold stage of freezing, suggesting little application of the latter in defining the product temperature in frozen state.

The results, during cooling and thermal equilibration post freezing, suggest that by exploring the slope functions for a particular product then values for $\log f_{\text{peak}}$ can be applied to calculate the product temperature with a reasonable degree of accuracy. A linear relationship was recorded between the $\log f_{\text{peak}}$ and the product temperature during both the pre-freezing and thermal equilibration stages (Fig 17).

The thermal coefficients for $\text{Log } f_{\text{peak}}$ are different in the two states (liquid and solid), with the solid phase displaying a greater temperature sensitivity than the liquid phase.

At this point it may be concluded that the f_{peak} shows more sensitivity to temperature and less sensitivity to the phase changes and vice versa for the C''_{peak} .

3.2.2 Peak Profiles during annealing (Phase II to III)

The temperature hold stage of the annealing period resulted in the stabilization of the pseudo-relaxation peak within a narrow frequency range (Fig 18). There is some evidence (though not conclusive at this stage) that the values from the thermocouple stabilise before those measurements from the impedance measurement system. This might point to the fact that the completion of the recrystallization phenomenon (Ostwald ripening) may occur after the system has reached thermal equilibrium.

More definitive evidence to suggest that the system has changed following the temperature ramp of the annealing stage comes from the temperature gradients in the $\log F_{\text{peak}}$ vs temperature plots, in that the slope factor for the temperature ramp 'up' was found to be ~ 15% higher than the annealing temperature ramp 'down' (Fig 19); There is also an apparent increase in $\text{Log } F_{\text{peak}}$ values on the ramp down phase compared to the ramp up phase. Both phenomena require further investigation as to its significance in terms of the structure of the frozen matrix.

3.2.3 Peak Profiles during primary drying (III to IV)

The impedance profiles (both the C''_{peak} and f_{peak}) changed significantly during the primary drying (Fig 20). The amplitude of the pseudo-relaxation peak decreases by a factor of ~ 100 during primary drying. Following a lag of approximately 2 h, from the onset of primary drying, the values for $\log C''_{\text{peak}}$ begin to decline in a linear fashion (Fig. 20b). This is a probable consequence of the drying front dropping below the top level of the measurement electrodes.

However, during the terminal stage of primary drying the pseudo-relaxation peak appears to be flat and wide. This may be seen in the 23h time point spectrum (Fig. 14) and in the 3-D surface plot (Fig 9a) whereby some low frequency noise results in the apparent broadening of the spectrum on the low frequency side of the peak.

At this point (> 20 h) the data analysis software begins to 'locate' the peak at a progressively lower frequency, hence the apparent decrease in the $\log f_{\text{peak}}$ vs time profile (see dotted line on Fig 20a) The low frequency noise also disturbs the estimation of the peak magnitude such that the $\log C''_{\text{peak}}$ vs time profile becomes noisy at time points beyond 20h (see dotted line on Fig. 18b). These uncertainties preclude the use of the pseudo relaxation peak in determining the end point of the primary drying phase.

It may be concluded that, while the peak vs time profiles are of value in the measurement of temperature changes and in characterising the various stages of the freezing step, an alternative approach is required for the determination of the end point of primary drying.

3.3 Time Slice Profiles at Fixed Frequencies

An alternative approach may be realised by use of time slices of the imaginary capacitance at some discrete frequency in the range 500-1000 Hz (i.e. the predominant location of the pseudo-relaxation peak during the primary drying stage). Fig 21a shows time slices at three frequencies of 500, 800 and 1000Hz. The time slice profiles for C' showed a reduction of only $\sim 2\%$ over the 7h period (20-27h) prior to the product-temperature-indicating end point. In addition there was no clear plateau at the end

of primary drying rather a minimum in C' was observed at ~23 h (fig 19 b). In contrast, the magnitude of C'' (1 kHz) decreased by ~ 70% over the same period (7 h) leading to an asymptotic value, suggesting the complete depletion of ice and the end point of primary drying.

Of the three frequencies profiled, a frequency of 1 kHz was considered to be optimal as it is well above that region where the low frequency noise impacts the signal quality Fig 21b.

Whilst it was anticipated that the values of C'' would approach a zero value as the primary drying cycled completed, it was not obvious as to how the exact end point could be defined. In order to arrive at a magnitude independent parameter for the determination of the end of primary drying, values for the derivative of the real and imaginary capacitance were then calculated as a function of time, i.e. $dC'_{1\text{kHz}}/dt$ and $dC''_{1\text{kHz}}/dt$. Log values of each derivative displayed a constant slope during the initial phases of primary drying, followed by a sharp decline at some time point (Fig 22). In the case of the log of the derivative of the real part capacitance, this transition point was observed at ~23.5 h (Fig 22a), whereas the transition point for the corresponding parameter for the imaginary capacitance ($\log dC''_{1\text{kHz}}/dt$) showed a sharp decline at ~27.5 h. In each case the transition point was estimated by measuring the intercept of the straight lines extrapolated from the slopes either side.

Of the two transition points, it was that for $\log dC''_{1\text{kHz}}/dt$ (27.5 h) which agreed with the thermocouple defined end of primary drying (at 27.2 h), i.e. the time at which product temperature increases above the shelf temperature (Fig 22c). However, this preliminary result demands further study. It is often considered that the time point at which the product temperature reaches that of the shelf temperature occurs some minutes prior to the true endpoint. It is for this reason that the endpoint of the cycle is often defined by the temperature endpoint plus 30 minutes. Further studies using microbalance are required in order to validate the endpoint.

4 General Discussion

It has been observed that the log of the pseudo-relaxation frequency has a linear correlation with the product temperature, provided that there is no product phase change over the temperature range of

interest, and that there are different gradients for the pre-freezing and solidified stages of the freezing cycle. It may be possible therefore, to calibrate the impedance measurement system to provide a non-product-invasive temperature sensor. The specific calibration coefficients of the sensor are likely to depend on the composition of the product; in particular, the concentration of any electrolyte or the nature and concentration of the counter ion of a salt form of a drug. These factors are likely to have a significant impact the conductivity of both the frozen and unfrozen solutions, which in turn will define the magnitude of the pseudo-relaxation frequency. Other components within the product, especially those which impact the viscosity of the solution and those which influence the extent of ice formation and the structure of the frozen matrix will in turn affect the conductivity and hence the relaxation frequency. Having recognized this dependence, it is also possible that certain process parameters, the freezing rate in particular, could additionally impact the structure of the ice matrix and therefore the conductivity of the frozen matrix. Whilst it remains to be seen whether general rules can be developed for the creation of formulation-specific calibration routines (which are independent of the process variable) even without calibration, it's clear that the use of impedance measurements could be applied for the measurement of hot and cold spots across the shelf, through measurements taken on discrete vials across an individual shelf.

The main purpose of this research article, however, was to establish the (i) utility of impedance spectroscopy in determining the characteristics of the freezing process, (ii) the end point of primary drying.

On freezing, it was shown that the measurement of impedance faithfully reproduces the observations made by a thermocouple, in terms of the onset time for ice formation, the duration of both solidification phase and the equilibration phase when the product temperature equals that of the shelf temperature. Experience has shown that there can be a wide range of freezing onset times within a freeze drier that contains a full load meaning there can be as much as 30 minutes difference between the ice formation in the first and last vial to undergo the freezing process. With the current study using the impedance system, where there were only five vials placed in close proximity to one another, one might expect less difference in ice onset time. However, we may expect to see differences that result

from the insertion of the thermocouple within the product, as it is known to provide additional nucleation sites, with an anticipated impact on the ice onset time. Visual observations by time lapse photography indicated that the thermocouple-containing vial nucleates and form ices approximately 120 s earlier than the four other vials. This time frame is within the 5 minute measurement cycle time to complete the scan on all five vials and therefore such differences between thermocouple containing vial and the impedance measurement vial are not picked up by this technique.

An accurate estimation of the end point of primary drying, the most critical step, has been a primary objective of the process development scientists. Conventionally, the endpoint of the primary drying has been predicted using thermocouple data as the time point at which product temperature raise back to the set shelf temperature. In this study we have used the conventional end point as a way of defining which of the transitions in the derivative of the imaginary capacitance time plot may be used as an end point indicator.

So far, it has been shown that the impedance measurement system gives analogous data to that of a thermocouple. However, the principle advantage of the impedance measurement system, over a simple thermocouple measurement, is that it can provide information on the rate of drying (as well as to highlighting the endpoint) of primary drying. Such information is not provided by the thermocouple since the temperature of the product stays largely constant during primary drying. In addition, the potential variations in the shape of the drying front (from planar to convex) can lead to inaccuracies in the measurement of sublimation endpoints, especially if the probe is positioned near the glass wall (Song et al., 2005). In contrast we have shown that the magnitude of imaginary capacitance (for example at a discrete frequency of 1 kHz) has a pronounced dependency on the extent of sublimation from the entire volume occupied by the product, with the potential to yield a definitive end of primary drying, free from probe related errors. So if we understand the relationships between the magnitude of imaginary capacitance and the composition of the product, in terms of the weight fraction of the residual ice, it might be possible then to predict the end point of the cycle well in advance of the actual endpoint. This would provide significant advantage to the process scientist who could then interrupt a cycle in advance of the cycle end point, thereby reducing the waiting time to assess the

actual duration of the primary drying stage. This would then accelerate the development process, by increasing the throughput of experiments within the Quality by Design approach to product and process development.

5 Conclusion

It has been demonstrated that the frequency of the dielectric pseudo-relaxation peak (f_{peak}) is strongly coupled to the temperature of the product (through the interdependency of the product temperature on the electrical resistance of the product) whereas the amplitude of the pseudo-relaxation peak (C''_{peak}) is dependent on the phase behavior and the extent to which ice has sublimed away from the product, during the early phase of primary drying. It follows that the magnitude of C''_{peak} may be used to define a drying rate. However, if the end point of primary drying is required then one must switch to the characterization of the log of the derivative of the magnitude of the imaginary capacitance at some fixed frequency close to that of the pseudo-relaxation peak frequency, e.g. 1 kHz. The end point is then defined in terms of the inflection in the gradient of the time profile.

It is anticipated that the impedance measurement system described in this article may be employed as one part of a process control system, in which the freezing rate, the shelf temperature and/or a termination of the lyophilization process or a sub-stage of the lyophilization process can be controlled in response to the product parameters measured by the system.

Future work

Future publications will focus on the use of impedance measurements in the determination of eutectic formation and the glass transition, the calibration of the system for temperature (in a range of formulations) and for the progression of the primary drying cycle (using gravimetric measurements from a freeze-drying microbalance).

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