

An application for impedance spectroscopy in the characterization of the glass transition during the lyophilization cycle: The example of a 10% w/v maltodextrin solution.

Geoff Smith^{*}, Muhammad Sohail Arshad, Eugene Polygalov, Irina Ermolina

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^{*}Corresponding author

Leicester School of Pharmacy, De Montfort University, Leicester, LE1 9BH, United Kingdom

Abstract

Impedance spectroscopy has been used for the measurement of the glass transition of a 10% malto-dextrin solution contained within a glass vial, with externally attached electrodes. Features of the pseudo-relaxation process, associated with the composite impedance of the glass vial-solution assembly, were characterised by the peak amplitude, C''_{peak} , and peak frequency, f_{peak} , of the capacitance spectra and the equivalent circuit elements that model the impedance spectra (i.e. the solution resistance and solution capacitance) and monitored every 3 min during re-heating of the solution. The time derivatives of all four parameters studied, provided a glass transition in close agreement with DSC measurements ($-17\text{ }^{\circ}\text{C}$) and at a precision of $\pm 0.5\text{ }^{\circ}\text{C}$. The temperature dependencies of the solution resistance and peak frequency were then characterized with the Arrhenius and Vogel-Fulcher-Tammann fit functions, at temperatures below and above T_g , respectively. The energy of activation (below T_g) was estimated at $\sim 20\text{ kJ mol}^{-1}$ and the fragility index (I_f) of the glass forming liquid (above T_g) was estimated at 0.9. The significance of the fragility index to the development, optimization and control of the freeze-drying cycle is highlighted.

Keywords:

Glass transition temperature, electrical impedance measurements, freeze drying, process analytical technologies

1. Introduction

The development of an appropriate formulation and an optimized process cycle for freeze drying of drug products is based on the determination of a set of customized process conditions which take in account the specific temperatures that define the physical state(s) of the drug formulation [1]. For solutions containing glass-forming solutes, the critical temperature is the glass transition temperature (T_g'), whereas for solutions containing crystallizing solutes, the critical temperature is the eutectic temperature (T_{eu}) [2, 3]. The measurement of these events requires the physical characterization of the solution during freezing and re-heating through a specific temperature range [4].

Freezing of a solution invariably induces the separation of the ice phase from the solute phase; a phenomenon known as freeze concentration or cryo-concentration. For formulations with non-crystallizing solutes, the solution phase is transformed progressively into a viscous liquid, followed by a rubbery mass and then a rigid glass with the continuous decrease in the solution temperature. The transition from rubber to rigid glass corresponds to the solution viscosity changing from 10^{11} - 10^{13} Poise to as high as $\sim 10^{14}$ Poise [5].

The high viscosity of the glassy state is a desirable feature of a solution to be freeze-dried, as it provides sufficient strength to support the matrix structure once the ice is sublimed. Temperatures close to this transition (the glass transition temperature or T_g') are meant to define the upper temperature limit for the mixture during primary drying. Above these temperatures, the solution viscosity is often insufficient for the unfrozen matrix to support its own weight. Invariably this results in the collapse of the product as the temperature is increased further. The temperature, at which collapse occurs, is known as collapse temperature (T_c) and is now routinely assessed through the use of a freeze-drying microscope. Whilst this technology is ideal for that specific purpose it fails to provide any additional information concerning the structural properties of the glass forming liquid, including the strength of the glass and the concentration of water in the unfrozen fraction.

Heating the solution through the glass transition results in enhanced molecular dynamics (with significant changes in the degrees of freedom associated with vibrational, translational and rotational motions). These degrees of freedom

are manifest in changes to the rheological, thermal, electrical and mechanical properties of the solution [6]. It is interesting to note that the temperature dependence of the molecular relaxation times in the glassy state is Arrhenius; whereas, at temperatures above the glass transition the temperature dependence of the relaxation times conforms to VTF behaviour. The reason for this change in the temperature response is due to the fact that the degree of cooperativity increases as the temperature is increased through T_g .

The techniques that are invariably used for the measurement of the glass transition temperature are differential scanning calorimetry (DSC) [7] and electrical impedance measurements [3, 8].

Differential Scanning Calorimetry (DSC) Despite its widespread application in the materials characterization, there are certain drawbacks with this technique: (i) T_g' is often difficult to characterize for dilute solutions; (ii) Sometimes the glass transition is coupled with a relaxation endotherm and hence the solution has to be cycled through T_g to release this entrapped energy and thereby observe the glass transition more clearly; and (iii) Multiple endothermic discontinuities can be potentially misleading. However the main disadvantage of DSC is that estimates for T_g' may not replicate those experienced by the solution in the process of freeze-drying, owing to the fact that the amount of ice that forms on freezing (and hence the concentration of solute in the unfrozen fraction) is impacted by the inequity in of freezing rates associated with the different container geometry and product fill heights, compared with the relatively small volumes used in DSC analysis.

Electrical resistance The application of electrical impedance measurements, to characterize the freezing behaviour of solutions, dates back to the 1960s [9]. In the majority of cases the solution impedance was measured at a fixed frequency (e.g. 1 kHz) by means of a pair of pin electrodes placed securely in a cryostat measurement cell containing the solution. Changes in the mobility of delocalised charges (ions) in the frozen solution are reflected in the electrical impedance of the sample as the solution is heated through the glass transition temperature. However, given that the electrodes are in direct contact with the frozen solution then this may alter the ice nucleation sites during the freezing stage, which could easily impact the rate of freezing and the amount of ice which forms [3]. This would in turn impact the concentration of solute in the unfrozen super-cooled liquid phase and hence alter the glass transition temperature experienced by the product.

Although the glass transition is readily defined by off-line measurement technologies e.g. DSC, it is not possible to witness the glass transition with the current range of in-line process analytical technologies, such as resistive temperature devices RTD and thermocouples. And yet, as was pointed out above, the glass transition experienced by the solution within a glass freeze-drying vial and the same solution within the aluminium DSC pan may be different. Nevertheless, the product temperature profile is employed to confirm whether the temperature corresponding to the off-line glass transition has been achieved. However, due to the uncertainties as to whether the temperature of all vials within the batch has passed through the glass transition during the freezing process then an additional soak time is often included in the freezing stage to ensure that the vials located in the hot spots of the drier are transformed to a stable frozen glass. The other issue with the use of thermocouples is that the direct contact with the frozen solution may alter the ice nucleation sites during the freezing stage (in a similar way to that we have described for the direct contact between the solution and the electrodes used for impedance measurements) which could then alter the glass transition temperature experienced by the product.

Our previous publication described an alternative electrical impedance approach for the in-situ measurement of the liquid to ice phase transition and the sublimation endpoint during the freeze drying process [10, 11]. That work demonstrated that an electrical impedance approach, with the electrodes attached to the external surface of the freeze-drying vial, could be used at both atmospheric pressure and at the relatively high vacuum of a typical freeze-drier. This present work explores the application of that technology to the in situ measurement of the glass to liquid transition of a surrogate product formulation comprising 10% w/v maltodextrin, during shelf freezing in the glass tubing vials. The rationale for the use of maltodextrin as a model formulation includes (i) its previous application as a lyoprotectant in the protein freeze drying, (ii) previous measurements of the glass transition process [12, 13] and (iii) the fact that the glass transition of the DE16-19.5 grade ($T_g = -17\text{ }^\circ\text{C}$) is midway in the temperature range achievable for the freeze-drier used for this study (0 - 35 $^\circ\text{C}$).

There are two objectives to this work. The first objective is to establish which of a number of parameters derived from the impedance response of the object under test (i.e. a glass freeze-drying vial containing a glass forming aqueous solution of a solute) provides the most definitive assessment of the glass transition temperature. The

second objective is to model the temperature dependency of the impedance response using Arrhenius and VTF functions in order to demonstrate that in process measurements by impedance spectroscopy may be used to characterise the glass forming properties (e.g. fragility) of solutions contained within glass freeze-drying vials.

2. Materials and Methods

Maltodextrin DE 16-19 was purchased from Sigma-Aldrich (UK) and used as supplied in the preparation of the solution. Distilled water was obtained from all-glass apparatus.

A surrogate solution containing 10% w/v maltodextrin was prepared by dissolving the solute in distilled water. Aliquots of 3.0 ml were introduced to the impedance measurement vials (N=2) using a syringe and micro filter 0.2 micron pore size Minisart® Germany. One measurement vial (vial 1 Fig. 1) was connected to a high precision impedance analyser installed immediately outside the freeze drier HETO FD 08 (Demark). A cooling/re-heating cycle was then implemented according to protocol described in Table 1.

Table 1 details of the cooling/re-heating cycle

Stage	Temperature (°C)	Time (h)	Cumulative cycle time (h)
Freezing temperature ramp	-35	1	1
Freezing temperature hold	-35	5	6
Thawing temperature ramp	-5	2	8
Hold time	-5	4	12

Impedance measurements were taken during the entire freeze-thaw cycle, over a frequency range 10^1 - 10^6 Hz with the scan interval of 3 min. Briefly, the impedance measurement technology comprises an modified glass vial (Fig. 1), which is a standard 10 ml, clear glass, tubing vial (Schott) with an added electrode system, which consists of a set of identical stimulating/sensing electrodes (18 x 5 mm) each with a surrounding grounded guard electrode. The guard electrode prevents electrical current leak between the stimulating and sensing electrodes, over the outer surface of the vial. The electrode system is 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 measurement vial is connected to the high precision impedance analyser via coaxial miniature cables.

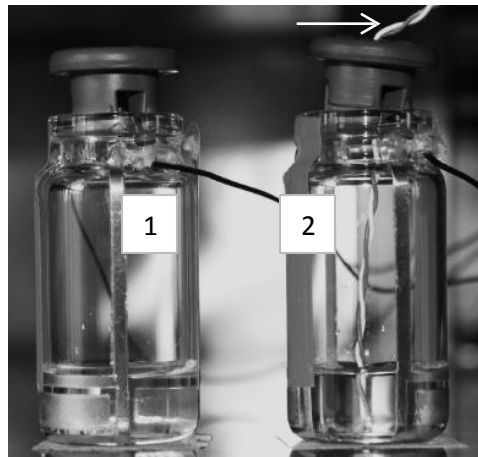


Fig 1 Linear Arrangement of the impedance measurement vials on the freeze drier shelf

Temperature measurements were recorded using a type K thermocouple, inserted in the second impedance measurement vials (vial 2, Fig. 1), at time intervals concurrent with the frequency of the impedance measurements (vial 2 Fig. 1). Provided there was no change in the physical state in the solution then it was possible to equate the solution temperature, measured in the neighbouring vial to that within the impedance measurement vial to within a limit of ± 0.15 °C. The reason why the impedance measurements were not recorded in the vial containing the thermocouple was that the presence of the non-grounded thermocouple corrupts the signal and disturbs the spectrum.

DSC analysis was also performed by scanning the maltodextrin solution (10% w/v) over a temperature range of -60 to 20 °C at rate of 10 C min⁻¹, using a Jade DSC (Perkin Elmer, USA).

3. Results

3.1 Differential Scanning Calorimetry

Thermograms of the solution of maltodextrin 10% w/v ($n = 3$) indicates an endothermic transition which can be described as a peak in the time derivative of heat flow, with an onset temperature of $-17 (\pm 0.5)^\circ\text{C}$, a mid-glass transition temperature of $-15.6 (\pm 0.5)^\circ\text{C}$ and end temperature of $-13.5 (\pm 0.3)^\circ\text{C}$ (one such thermogram is shown in Figure 2). The glass transition temperature (T_g') was in agreement with the value given by [14].

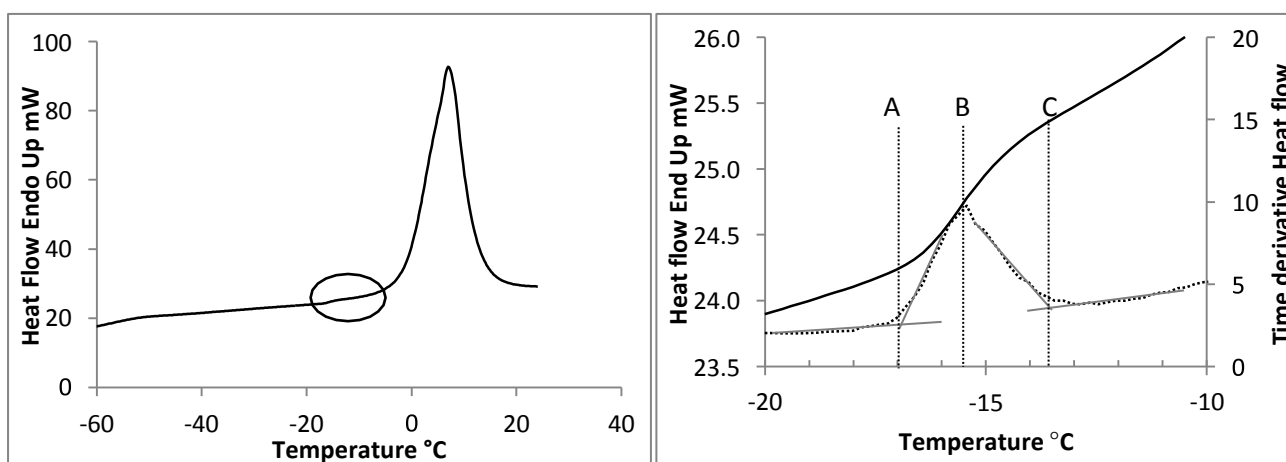


Fig 2 DSC results of Maltodextrin 10% w/v solution. Left: Complete thermogram (area circled shows the approximate location of the glass transition). Right: Expansion of scale to show the glass transition as a step in the heat flow curve and a peak in the derivative curve.

3.2 Impedance Measurements

The impedance measurement system records the physical changes of the solution in terms of the composite impedance of the electrode-vial- solution assembly. This composite impedance is responsible for the apparent pseudo-relaxation process which is observed in experimental frequency window of 10^1 to 10^6 Hz as a step in the real capacitance (C') spectrum and as a peak in imaginary capacitance (C'') spectrum (Fig. 3 A, B).

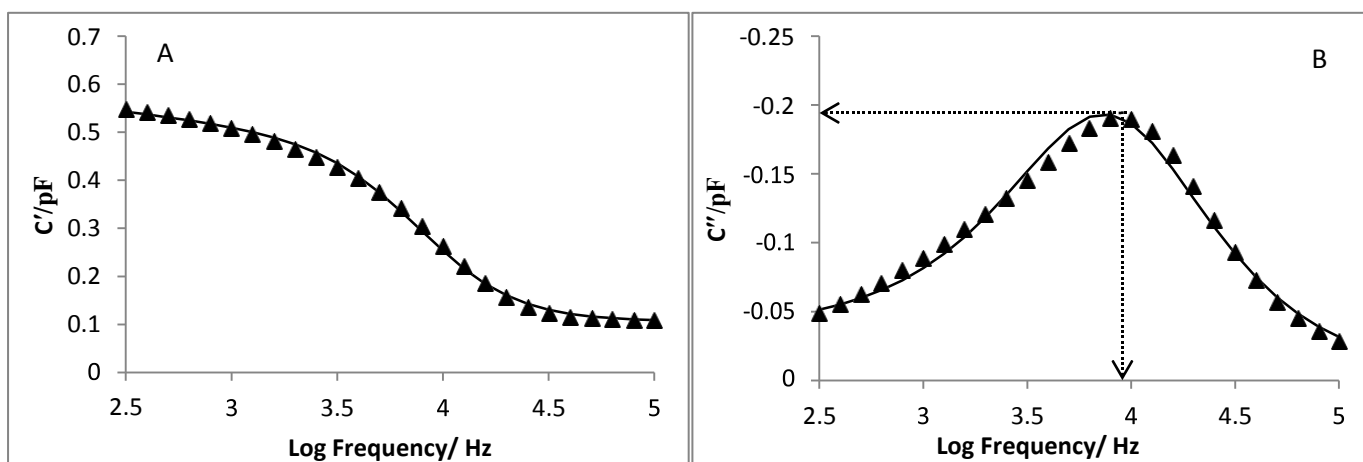


Fig 3 Capacitance spectrum of maltodextrin (10% w/v) at 270 min, when the solution had sufficient time to freeze solid. LEFT hand spectrum show the real part (C') and the RIGHT hand spectrum show imaginary part (C''). The triangle mark (\blacktriangle) show the experimental data. Solid lines (—) describe the fit results to the data using the impedance model described in Fig. 4.

Changes in the pseudo-relaxation process can be monitored empirically by assessing the changes in the peak amplitude (C''_{peak}) and peak frequency (f_{peak}) in the imaginary capacitance spectrum. Fig. 3B illustrates how the frequency position, f_{peak} , and peak amplitude, C''_{peak} are derived from the imaginary capacitance spectrum. It has been demonstrated in our previous publication [11] that the characteristics of this pseudo-relaxation process are dependent on the electrical conductivity of the formulation, which is in turn dependent on the product temperature and physical state of the formulation (i.e. whether the solution is in the frozen/solid state or the liquid state). Furthermore, the features of the peak (the frequency position, f_{peak} , and peak amplitude, C''_{peak}) have been shown to describe different stages of freeze drying process, i.e. temperature changes during the cooling of the solution, ice formation and sublimation.

In order to derive a more direct understanding of the relationships between the impedance response (i.e. the pseudo-relaxation process) and the physical characteristics of the solution, an equivalent circuit model was used to fit the capacitance spectra. This equivalent circuit comprises three physical elements: a constant phase element (CPE), a resistor (R) and a capacitor (C) (Fig 4); The interfacial impedance between the solution and the glass wall is defined by CPE while R and C describe the physical state of the solution (in terms of the conductivity and dielectric permittivity). An example fit result using this equivalent circuit model are shown in (Fig 3)

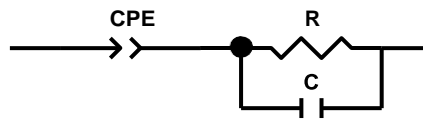


Figure 4. An equivalent circuit that accounts for the origin of the pseudo-relaxation process

Given that two methods are being presented to characterise the pseudo-relaxation process of the object under test, it is helpful to define the inter-relationships between the two methods: From the peak frequency one can calculate a time constant ($\tau = 1/2\pi f$) which characterises the rate of charging of the interfacial impedance of the glass wall of the vial. The rate of charging is related to 1) the diffusivity of charges to the interface between the solution and the glass wall and 2) the amount of charge that may accumulate at the interface. The former is reflected in the solution resistance and the latter is reflected in the magnitude of the interfacial capacitance between the frozen solution and the glass wall ($|CPE|$). The time constant is simply derived from the product of the solution resistance (R) and the capacitance of the constant phase element ($|CPE|$), i.e. $\tau = R \cdot |CPE|$. So by assuming that $|CPE|$ remains constant during the re-heating phase, it follows that there will be a direct relationship between R and f_{peak} , i.e. $f_{peak} \propto 1/R$.

Having defined these relationships, it's then possible to explain the changes in the peak frequency position in terms of the physical changes to the solution during the freezing and re-heating phases of the cycle. Fig. 5 shows the temperature profile of the near-neighbour vial (Fig. 1; vial 1) and demonstrates the four phases of super-cooling (A to B); freezing (B-C), temperature equilibration with the shelf (C-D), and the re-heating or annealing phase (D to E). The corresponding changes that one can observe for the capacitance spectra are shown in Fig. 6.

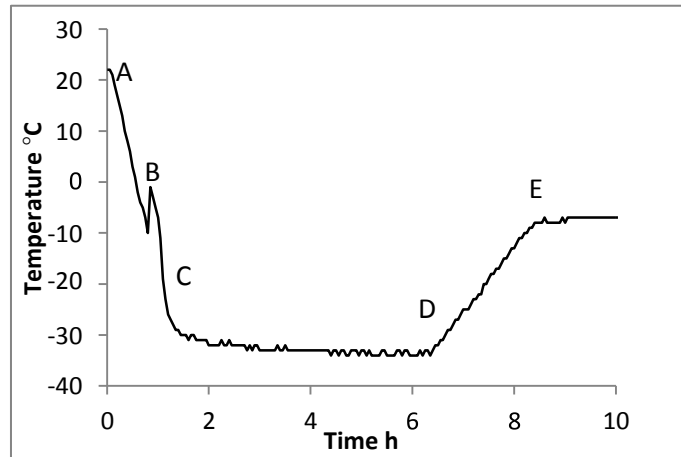


Fig.5 Temperature profile of maltodextrin 10% w/v during cooling and re-heating

In the super-cooling phase (Fig. 5 region A-B) the pseudo relaxation peak of the maltodextrin solution remains outside (i.e. the right of) the measurement frequency range (Fig 6 A,B), primarily because of the high solution conductivity (high conductivity translates to a low resistance, a fast time constant and therefore a high peak frequency). It was therefore not possible to document the frequency position, f_{peak} , and peak amplitude, C''_{peak} for this formulation in the unfrozen state. However, in the ice formation stage, the solution viscosity increases dramatically and so does the electrical resistance of the solution. The peak frequency then decreases and brings the pseudo-relaxation peak within the experimental frequency range (Fig. 6 C,D). In the ice nucleation and growth region (Fig. 5 region B-C), the precipitation of ice concentrates the unfrozen fraction (containing maltodextrin), the viscosity of the system increases and the number of mobile charge carriers decreases. The overall result is a rapid increase in solution resistance and a shift in the pseudo-relaxation frequency so that it appears in the experimental frequency range (Fig. 6 C and D). Finally, during the re-heating of the solution (Fig. 5 region D-E) the increase in temperature decreases the electrical resistance of the solution and the pseudo-relaxation process shifts to higher frequencies (Fig. 6 E and F).

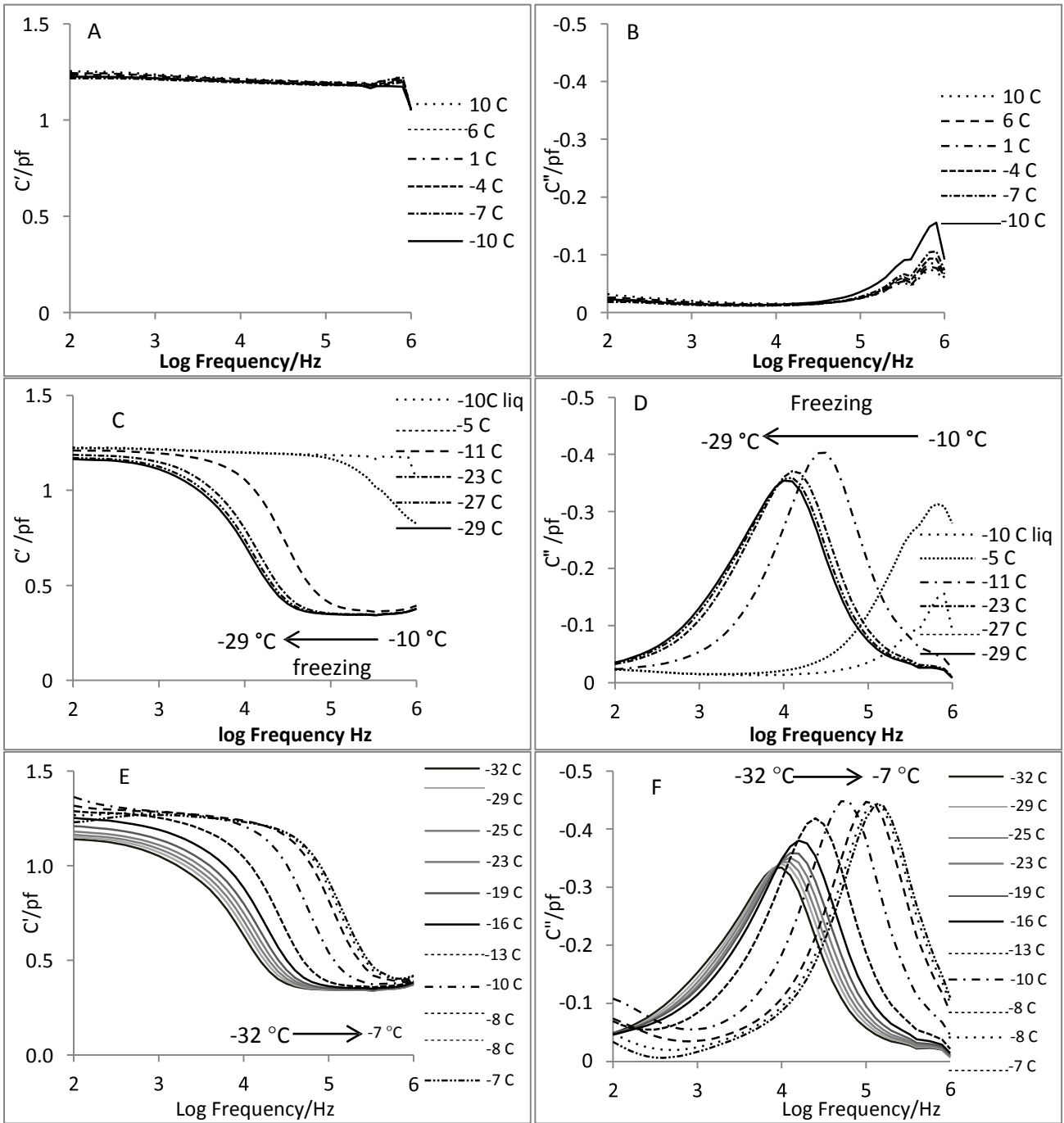


Fig. 6. Capacitance spectrum of maltodextrin (10% w/v) in liquid state (Left side shows the real part capacitance and the right side shows the imaginary part capacitance); The pseudo-relaxation peak exist above the measurement range, and frozen states (c, real part and d, imaginary part); the pseudo relaxation peak were recorded during thawing of the frozen solution from -32 °C to -7 °C.

3.2.1 Determination of the glass transition temperature

The objective of this part of the study is to establish which of the four parameters derived from the impedance response of the freeze-drying vial, containing a solution of glass forming solute (i.e. the peak frequency, f_{peak} , and peak amplitude, C''_{peak} from the imaginary capacitance plot, and the estimates for the solution resistance and solution capacitance, R and C, respectively) provides the clearest assessment of the glass transition temperature on re-heating the solution following the initial freezing stage.

Three approaches to the registration of the glass transition, or more precisely the glass to liquid transition (GLT), are described. The first is the manifestation of the GLT in the time profiles for the four parameters of interest (i.e. the f_{peak} , C''_{peak} , R and C); the second is the manifestation of the GLT in the temperature profile of the same four parameters (i.e. where each parameter, as determined from the impedance response of vial 2, is plotted against the temperature recorded in vial 1); and the third is the manifestation of the GLT in the time derivative of each of the four parameters, plotted against the temperature recorded in the neighbouring vial.

In all three approaches, each of the four parameters (f_{peak} , C''_{peak} , R and C) demonstrated an inflection in the profile, which is taken to be the glass transition of the solution. In general terms, the inflection in each parameter may be explained by the increased mobility of the molecules/ions following the second order phase transition from the glassy state to viscous rubber and liquid. For each approach, a simple method of 'intersecting straight lines' was used to extract a 'definitive' assessment of T_g from the four parameters. This method assumes (rightly or wrongly) that the data above and below the transition can be taken as linear. The data was partitioned into two sets (i.e. data above and data below the transition temperature) based on the visual assessment of the apparent transition temperature; and the lines of best fit to each data set calculated by linear regression. By equating each line of best fit to one another it was then possible to estimate the transition temperature.

The change in the gradient of each best fit line was then used as an index of the potential discrimination that each parameter has for observing the glass transition. In other words the greater the % change in the gradient the more likely it is that the parameter in question would be able to observe the glass transitions.

Time Profiles for f_{peak} , C''_{peak} , C and R The parameters derived from the peak analysis (f_{peak} and C''_{peak}) and impedance modelling (R and C), and the temperature recorded by the thermocouple, are shown in Fig. 7 as a function of time elapsed through the re-heating stage of the freezing/annealing cycle. The first observation to note is that while the temperature rise is linear with time as the product temperature was raised from -32 to -7 °C, all the parameters from the pseudo-relaxation process show discontinuities in the time profile at the ~ 8h which correspond to the temperature passing through the range -14 to -17 °C (Table 2).

Both the f_{peak} and C''_{peak} parameters have an inflection point at 7.9 h whereas the impedance modelling parameters R and C have an inflection point at 7.75 h, which correspond to the temperatures -14 °C and -16 °C respectively (Fig 7 a, b, c and d respectively). Estimate for the GLT R and C were therefore in closer agreement with the DSC results than f_{peak} and C''_{peak} .

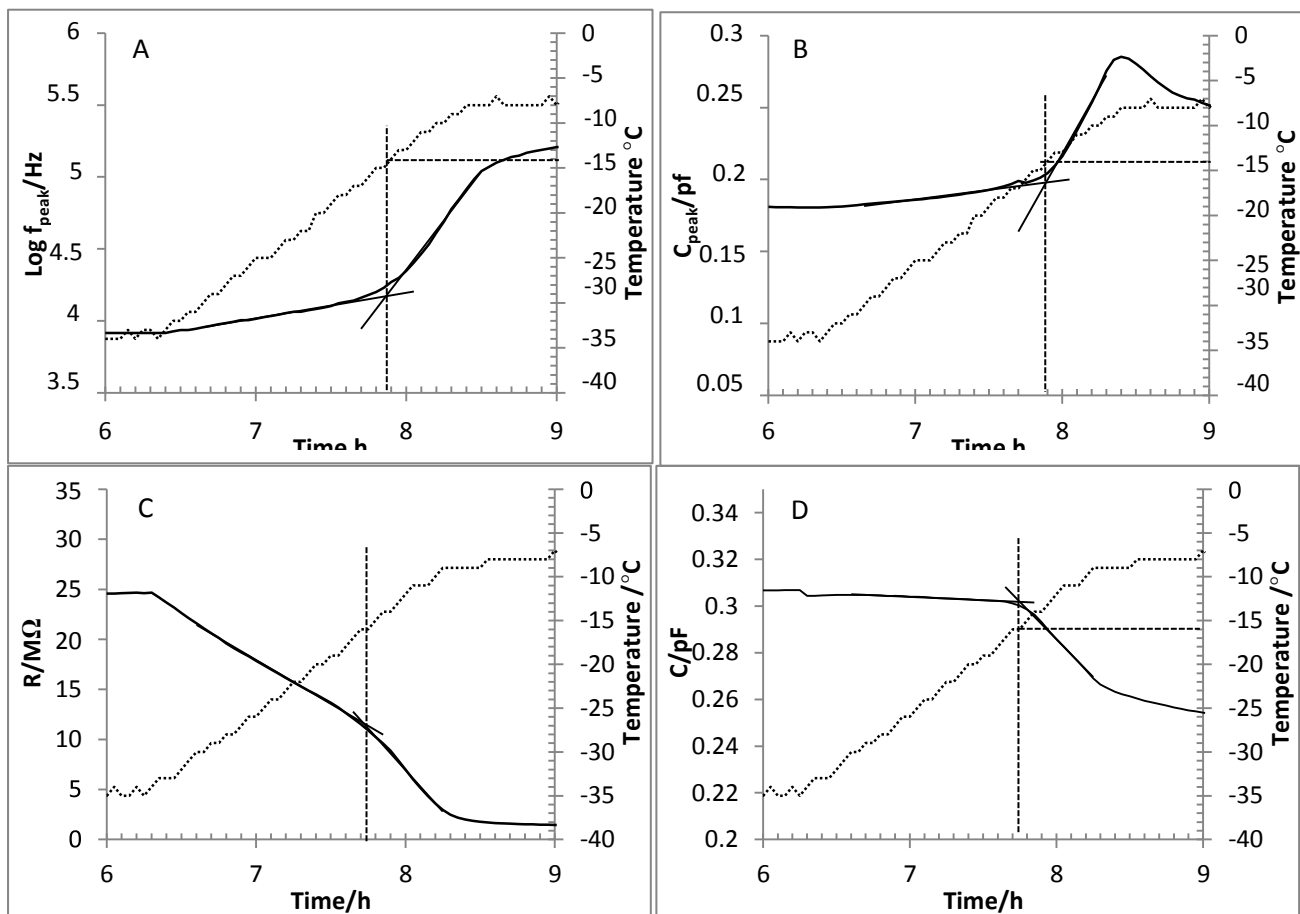


Figure 7 Time profiles of parameters (A) f_{peak} (B) C''_{peak} (C) R and (D) C from spectral analysis of a 10%w/v aqueous solution of maltodextrin during re-heating the solution from -32 °C to -7 °C.

Table 2 Regression line parameters for the lines of best fit to the time dependencies of the spectral peak analysis and impedance modelling parameters, at temperatures below and above T_g

	Inflection Time (h)	Below T_g			Above T_g		
		slope	intercept	R2	slope	intercept	R2
f_{peak}	7.9	0.1779	2.7715	0.9928	1.3666	6.578	0.9935
C''_{peak}	7.9	0.0131	0.0947	0.966	0.1807	-1.2273	0.9902
R	7.75	-8.7571	79.22	0.9992	-16.623	139.95	0.9973
C	7.75	-0.0028	0.3238	0.9852	-0.0643	0.8001	0.9956

Temperature Profiles for f_{peak} , C''_{peak} , C and R In order to be clearer about the specific temperature at which the apparent glass transition is observed, the parameters derived from peak analysis (f_{peak} and C''_{peak}) and impedance modelling (R and C) were then plotted as a function of the temperature recorded by the thermocouple in the 1st vial (Fig. 8). As with the time profiles, all parameters show a discontinuity in the rate of change at some temperature in the range -14 to -16 °C (Table 3), which again corresponds to the glass transition as measured by DSC.

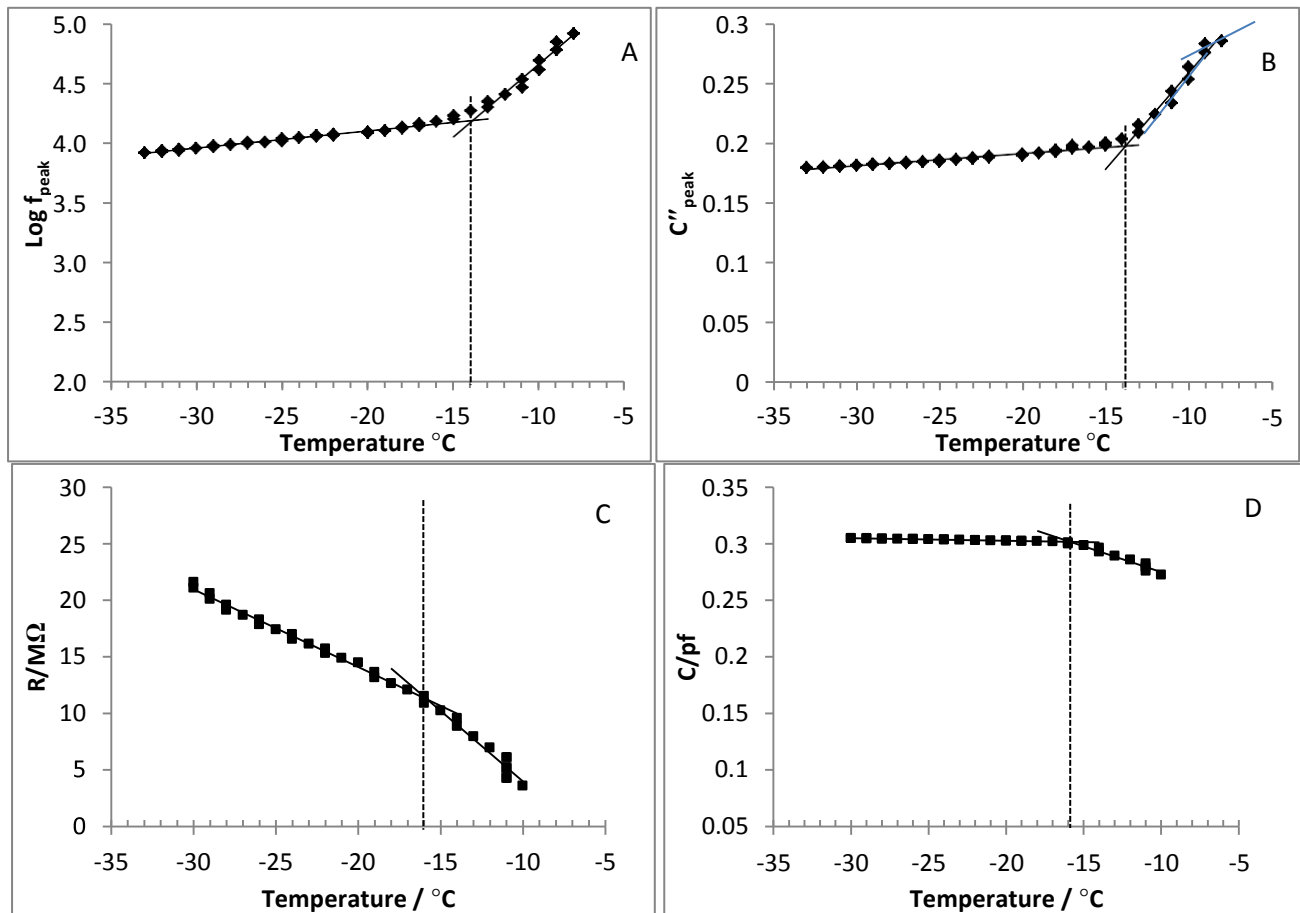


Figure 8. Temperature profiles of parameters (A) f_{peak} (B), C''_{peak} (C), R and (D) C from spectral analysis of a 10%w/v aqueous solution of maltodextrin during re-heating the solution from -32 °C to -7 °C.

Table 3 Regression line parameters for the lines of best fit to the temperature dependencies of the spectral peak analysis and impedance modelling parameters, at temperatures below and above T_g

Parameter	Inflection temperature (°C)	Below T_g			Above T_g		
		slope	intercept	R2	slope	intercept	R2
f_{peak}	-13.9	0.0145	4.3883	0.9865	0.1228	5.8893	0.9618
C''_{peak}	-13.8	0.001	0.2126	0.9568	0.0159	0.4172	0.9623
R	-16.0	-0.687	0.3682	0.9916	-1.2482	-8.4818	0.9655
C	-15.8	-0.0002	0.2992	0.9833	-0.0046	0.2291	0.9528

The derivative profiles of f_{peak} , C''_{peak} , C and R The time derivatives of the impedance parameters f_{peak} , C''_{peak} , and the impedance modelling parameters C and R were calculated from the slope of the time dependent response over a period of 0.5h. This routine was continued through the entire cycle. As above, the temperature values corresponding to glass transition were estimated from the intersection between the line of best fit, above and below the inflection points. What is interesting to note is that the gradient above T_g continues to change with temperature, which means that the temperature profiles for each parameter above T_g are not linear, as assumed in the analysis above. This may explain why the inter-section of the two lines of best fit provide an estimate for T_g which are, in general, lower than that determined by DSC.

The glass transition temperature derived from time derivative data are summarized in Table 4. The T_g value calculated from time derivative of C''_{peak} , f_{peak} and element C were ~ -17 °C whereas that from element R was ~ -19 °C (Fig. 9). Therefore, of the three approaches to the determination of the glass transition, it was the third method using the plot of the derivative of the pseudo-relaxation parameters (C''_{peak} , f_{peak} , R, C) that provided the closest estimates to that determined by DSC.

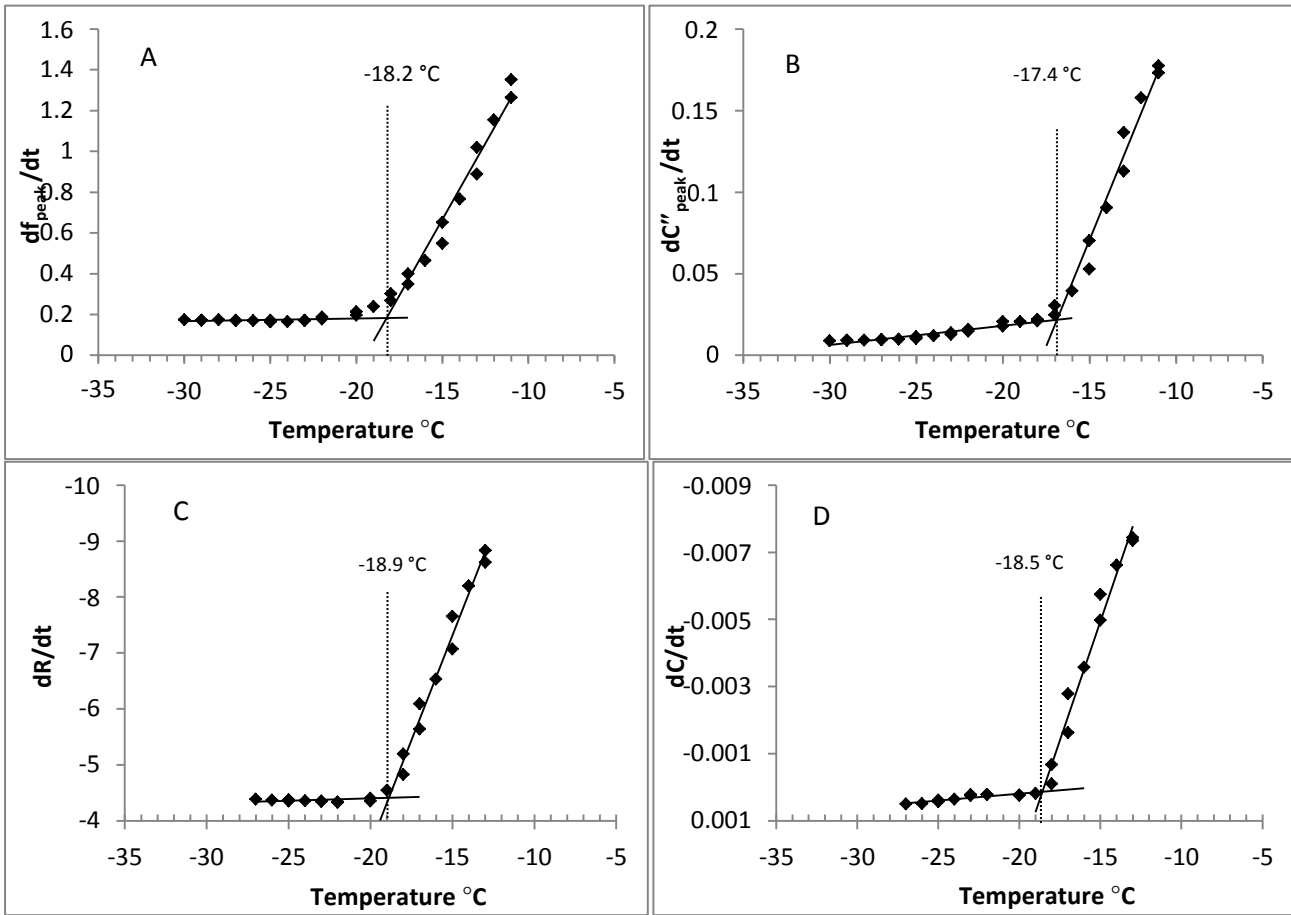


Fig 9 Time derivative profiles of parameters (A) f_{peak} (B) C''_{peak} (C) R and (D) C from spectral analysis of a 10%w/v aqueous solution of maltodextrin during re-heating the solution from -32 °C to -7 °C.

Table 4 Regression line parameters for the lines of best fit to the time derivative of the spectral peak analysis and impedance modelling parameters, at temperatures below and above T_g

Parameter	Inflection temperature (°C)	Below T_g			Above T_g		
		slope	intercept	R	Slope	intercept	R
$d\log f_{\text{peak}}/dt$	-18.2	0.0014	0.2075	0.2342	0.1492	2.9046	0.9721
$dC''_{\text{peak}}(\text{pF})/dt$	-17.4	0.0012	0.0416	0.9078	0.026	0.4611	0.972
dR/dt	-18.9	-0.0089	-4.5773	0.1596	-0.07479	-18.579	0.9777
dC/dt	-18.5	-0.00004	-0.0006	0.8172	-0.0014	-0.0262	0.9698

Having established that the derivative plots provide estimates for T_g which are closer to those determined by DSC, the question remaining is which parameter (dF_{peak}/dt , dC''_{peak}/dt , dC/dt , or dR/dt) provides the most reliable assessment of the glass transition temperature. Two ways of making this judgment were proposed: The first was to assess the % change in the gradient between the line of best fit above T_g and the line of best fit below T_g , whereby the denominator in this equation was taken from line with the largest gradient. In all cases this was the gradient from the line of best fit to the data above the glass transition T_g (eqn 1).

$$\% \text{ change} = 100 \times (\text{gradient above } T_g - \text{gradient below } T_g) / \text{gradient above } T_g \quad (1)$$

The percentage change in the gradient of regression line can be considered as one indication of the sensitivity of that parameter to the glass transition and hence the potential resolution afforded.

The second approach was to use the root mean squared error for each line to determine the uncertainty in the estimate for T_g . In this method the upper limit for the uncertainty in T_g was calculated by adding the RMS error to the value of the intercept for the sub T_g line and subtracting it from the line of best fit for the intercept for the supra- T_g line (fig 10 B). The lower limit was calculated in an analogous way.

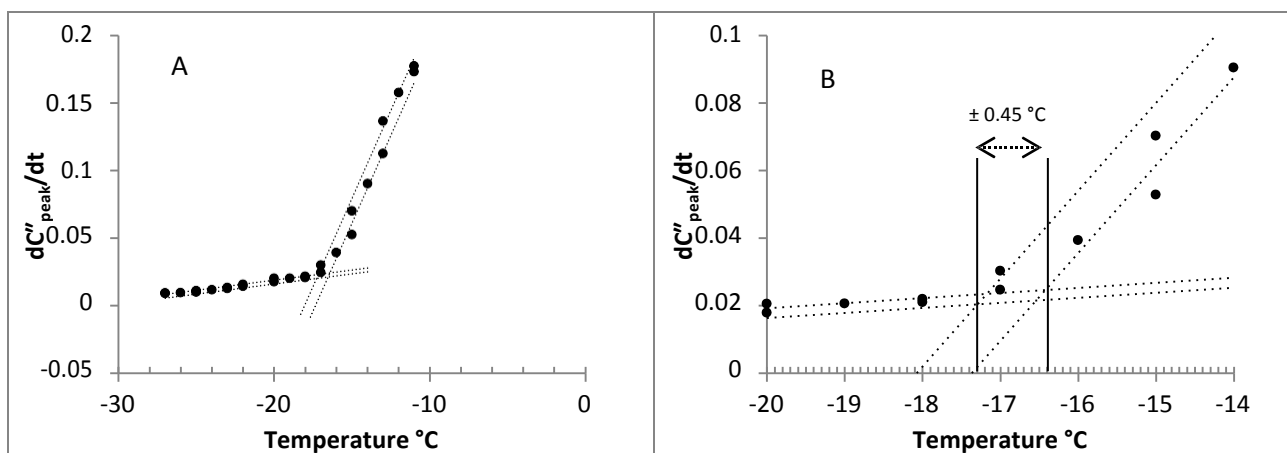


Figure 10 Temperature profile of dC''_{peak}/dt to illustrate the methodology for calculating the uncertainty in T_g from RSM errors. (A) full data set; (B) reduced data set close to T_g . Dotted lines show the rms error between the line of best fit and the experimental data.

This assessment of the degree of uncertainty can be taken as a measure of the precision of the estimate of glass transition.

The rank order of parameters for % change in the gradient following the glass transition was observed as $f_{\text{peak}} \sim C \sim C''_{\text{peak}} > R$, whereas the rank order of parameters for the uncertainty in the inflection temperature was $C \sim R < C''_{\text{peak}} < f_{\text{peak}}$.

Table 5. Percentage increment in the slope of regression line of time derivatives of parameters f_{peak} , C''_{peak} , R and C following glass transition

Parameter	% increment in slope	(Inflection temperature, T_g ; °C)	Uncertainty in the inflection temperature (\pm °C)
$d \log f_{\text{peak}} / dt$	99	-18.2	0.5
$d C''_{\text{peak}} (\text{pF}) / dt$	95	-17.4	0.45
dR / dt	88	-18.9	0.3
dC / dt	97	-18.5	0.3

3.2.2 Characterization of the glass forming liquid, above and below T_g

Of the four parameters that model the impedance response, it is the product resistance (R) which reflects the charge transfer processes through the frozen solution. The origin of the charge, in a frozen aqueous solution are likely to be the protons which are liberated from water and then hop from one hydrogen bond to another [15].

As, explained earlier, there is a direct relationship between R and f_{peak} (i.e. $f_{\text{peak}} \propto 1/R$) and so the parameter f_{peak} may also be used to track changes in the physical state of the frozen solution. However, this is predicated on the requirement for |CPE| to remain constant during the annealing phase. Fig. 11 shows the temperature dependency of |CPE| during the annealing phase. The change in |CPE| over the VTF temperature range is $\sim 9\%$, thereby questioning whether this parameter may be considered as a constant over the range of temperatures investigated.

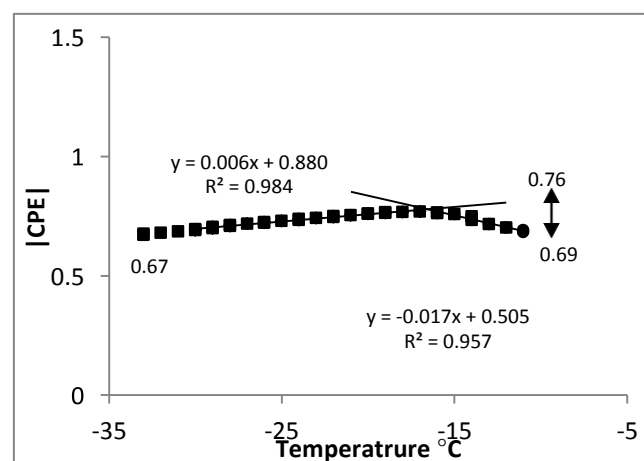


Fig 11 Temperature profile of impedance modelling element CPE

Changes to both R and $1/f_{\text{peak}}$, over the temperature ranges either side of the glass transition temperature, were then characterized by the mathematical expression for Arrhenius behaviour and the simplified expression for Vogel Fitcher Tammann (VTF) behaviour, (Eqns 2 and 3 respectively) in order to understand the physical behavior of the solution in both the glassy and liquid states.

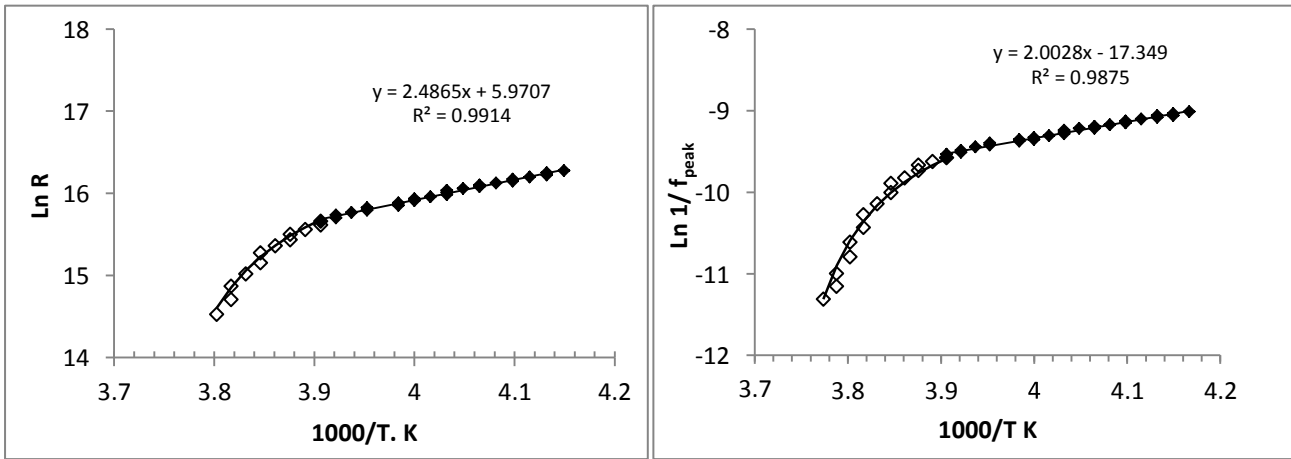


Fig 12 Arrhenius plot showing the temperature dependence of the resistance element R and the peak frequency (f_{peak}) for 10% w/v maltodextrin

$$\ln A = \ln A_0 - E_a/RT \quad (2)$$

In Eqn 2, A_0 is pre exponential value, E_a is energy of activation ($\text{Joule}\cdot\text{mol}^{-1}$), R is the gas constant ($8.314 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$), and T is the temperature in Kelvin.

$$\ln A = \ln A_\infty + B/(T-T_0) \quad (3)$$

In Eqn 3, A_∞ refers to the pre-exponential product resistance and f_{peak} at low temperature, B is the constant relating to the diffusivity of molecules (or charge carriers in our case) and T_0 is a constant known as the Vogel temperature. In effect the VTF function is modelling the non-linearity in the Arrhenius plots for resistance (R) and peak frequency (f_{peak}) above T_g .

From the Arrhenius plots of f_{peak} and R , the energy of activation below T_g was found to be 17 ± 1 and $22\pm 1 \text{ kJ mol}^{-1}$ respectively (Fig 12). The latter was in close agreement with the activation energy reported for maltodextrin solutions using electron spin resonance (ESR) spectroscopy [16]. The activation energy in the sub- T_g region has been ascribed previously to hydrogen bond dissociation [17] and can be considered as the average strength of a hydrogen bond in the unfrozen fraction. The fact that f_{peak} provides a slightly lower estimate for activation energy might

suggest that the earlier assumption of CPE remaining constant over the measured temperature range may not be strictly accurate.

The fit parameters for the VTF function are given in Table 6. Close agreement between the estimates for B and T_0 suggest that either method could be used to characterise the unfrozen fraction above the glass transition temperature.

Table 6 VTF fit results of Maltodextrin 10% w/v solution

parameter	Ln A_∞	B	T_0 (K)
R	16.88	-0.265	271.2
$1/f_{\text{peak}}$	-8.5	-0.235	271.0

The fragility index (I_f) was defined by the equation

$$I_f = T_0/T_g \approx 1 - B \cdot R/E_a \quad (4)$$

Where B is VTF fit parameter, R is universal gas constant and E_a is the energy of activation from the Arrhenius fit results. The index suggest value of $I_f = 0$ for strong glasses while > 0.6 describe fragile glasses. For maltodextrin solution, the I_f value was ~ 0.9 , which suggests a fragile nature of the frozen glassy solution [18].

Another empirical approach for estimating the fragility parameter above the glass transition is to calculate the slope (m) of Ln R and f_{peak} between T_g to $T_g/2$ (Fig. 13)

$$\text{Slope (m)} = d \text{ Ln R} / d(T/T_g) \quad (5)$$

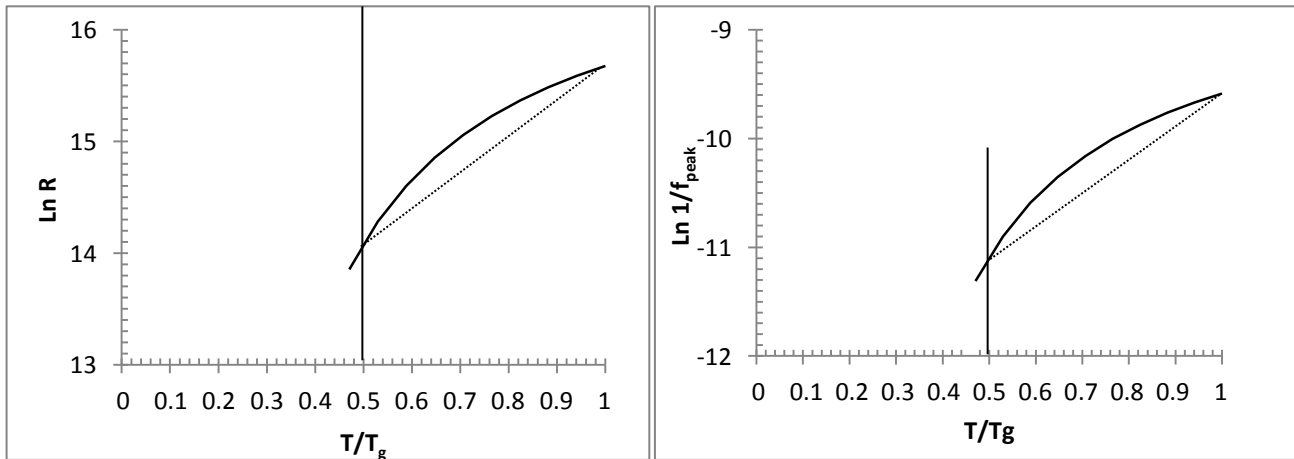


Figure 13 – VTF fit line above T_g for 10% w/v maltodextrin

The slope (m) for both parameters R and $1/f_{\text{peak}}$ was recorded as 0.75.

4. Discussion

Different methodologies are described for analysing the pseudo-relaxation peak arising from the composite impedance of the solution-glass-wall assembly of a liquid filled freeze-drying vial, with a view to defining the glass to liquid transition of a 10% solution of malto-dextrin. The derivative of the time profile of a number of parameters (f_{peak} , C_{peak} , R and C) were found to give the closest estimate to the DSC value ($-17\text{ }^\circ\text{C}$) with a precision of ($\pm 0.5\text{ }^\circ\text{C}$). The exploitation of this understanding will become most relevant when the in-vial impedance measurement approach is extended to the determination of the glass transition in more dilute solutions. Although not reported here we have also demonstrated that the same methodology may be used to characterise glass forming solutions of poly vinyl pyrrolidone (PVP) and lactose.

The in-situ impedance measurement described in this article may be more suitable than a number of off-line techniques, e.g. DSC and electrical impedance, as the latter are expected to measure a T_g which is different to that experienced by a solution frozen in the vial, due to the fact the former freezes at a comparatively high rates and that the container geometry and sample volume are very different. These features of the off-line techniques could

provide a greater degree of super cooling and result in a lower concentration in the unfrozen fraction and therefore a higher glass transition temperature.

The accurate determination of T_g on the production line may have some relevance to process control strategies in that it may enable the operator to drive the primary drying stage at a higher temperature than is permitted by convention. By convention, the shelf temperature during the freezing stage is usually set at $2-3\text{ }^\circ\text{C} < T_g'$ in order to allow for the thermal loss associated with glass vial base. And since a thermocouple probe does not evidence any visible change in the temperature profile following a second order transition (such as the glass transition) then these probes are simply employed to register with the previously measured temperatures (off-line tools) corresponding to the glass-liquid transition of the solution.

This margin in temperature is also applied to real freeze-drying processes because one cannot be sure about the spatial distribution of temperature in the drier, given that there is a thermal lag between the shelf and the vial contents due to the poor thermal contact between the shelf and the vial base. The advantage of a single vial measurement technique, such as that provided by the impedance technology proposed here, is that it may be used to monitor critical domains within the drier where hot and cold spots define the extremes of temperature experienced by the whole population of vials across the drier. A multi-channel approach to the proposed impedance measurement system could analyse a number of vials simultaneously and, hence it could be employed to record the glass transition at different spatial position across the shelf. This would provide a realistic soak time required to ensure the entire formulation units have approached glass transition.

The time derivative of the in situ impedance measurement could then be used to signal that all vials have passed through the glass transition temperature and that it is safe to continue from pre-freezing to primary drying. This would also deliver process efficiencies as it would enable the operator to drive the primary drying stage at a higher temperature than might be permissible if a safety margin of a few degrees in temperature had to be applied. Rather than controlling the process on the basis of temperature (with the safety margin applied) one could instead control the process based on a set point, one which is defined by the observation as to whether the population of vials have

their contents in the desired physical state. In this case the desired state is that the solution is in the high viscosity state of a rigid glass.

The physical properties of frozen glassy solution are explained in terms of the unfrozen fraction of the water, the freeze concentration of the solute and the size distribution of ice crystals. Below T_g , the observed Arrhenius behaviour suggests that a proton hopping phenomenon under pins the charge transport processes. Above T_g , a good correlation with the VTF function suggests a cooperativity in the motions of molecules following an increased free volume.

Various approaches were taken to determine the fragility parameter of the unfrozen fraction above T_g . All provide reasonable estimates which are similar to those found in the literature for aqueous solution of polymers [19]. The fragility parameter may have direct relevance to process development, especially in regard to the impact of annealing on the concentration of water remaining in the unfrozen fraction. A decrease in the slope factor (i.e. a tendency to become more linear) following annealing, may point to an increased strength of the glass (i.e. reduced fragility). The significance of the fragility to freeze-drying method development is that it may provide a useful index for the concentration of water in the unfrozen fraction, given that fragility is known to increase with increasing water fraction. Indeed water itself shows the highest fragility. The transformation of water from the unfrozen to frozen state may lower the risk of melt back or collapse during the drying stages. In addition, a conditioned (i.e. annealed) frozen product formulation may therefore dry quicker (in the secondary drying stage) than a non-annealed formulation. This may also have further implications for the lyophilisation of biopharmaceuticals whereby the concentration of protein in the unfrozen fraction could have a bearing on the destabilization of the protein through phenomena such as aggregation.

A more in-depth and mechanistic interpretation as to the impact of composition on the fragility of the glass forming materials have been proposed previously [20] which suggests that the phenomenon may be linked to the local heterogeneity in the dynamics of fragile glass formers. In binary systems, such as a super-cooled solution of maltodextrin, this might even suggest slow and fast regions which are either polymer rich or water rich respectively. It is outside the scope of this article to expand further on such possible interpretations.

At the beginning of the article, the relevance of the glass transition to maintaining a high solution viscosity and the avoidance of the collapse was highlighted. However, the problem with the measurement of collapse (whether it is by off-line freeze-drying microscopy or by an on-line technique such as extra-vial impedance measurements) is that there is no prior indication that collapse is about to occur. In other words, there is no inflection in any measurable physical parameter which might signify a pre-collapse microstate has been reached. In addition the thermal inertia of the freeze-drying process means that it would be almost impossible to reverse a temperature ramp that projected the frozen solution to a state of collapse. Instead, what may be possible is that there is a link between the fragility determined by the in situ impedance measurement and the propensity for a super-cooled matrix to collapse. This possibility will form the basis of future articles using impedance spectroscopy.

It has been shown that impedance measurements can be applied to measure the glass transition of the product contained in the glass vial without impacting the freezing process. This provides the rationale for the development of freeze drying cycles without the necessity of a safety temperature margin which makes allowance for the variation in thermal conditions across the shelf. By probing the spatial map of the thermal characteristics across the shelf using impedance measurements at critical positions, e.g. corners and centre of each shelf, one may be able to determine whether the batch has collectively passed through the glass transition during freezing. That would then enable one to set appropriate annealing conditions (set temperatures and holding times) which could then lead to the optimization of the process. This work has also demonstrated the use of in-situ measurement in characterizing the strength of glass forming solution above T_g and highlights the potential application in both formulation and process development for biopharmaceuticals in particular.

As the impedance measurement defines both the glass transition and primary drying end point, it follows that this technological approach may be employed in a multi-functional and non-invasive way: First to establish the freezing and primary drying temperatures, then secondly to investigate the optimum annealing temperature and to study its consequences in term of primary drying time in the real process conditions. In comparison with the conventional methodologies practiced for freeze drying method development/optimization (i.e. product characterization by off-line tools and product temperature measurement by invasive thermocouple probes), the impedance measurement

technology offers a reliable measurement of the critical product and process parameters and is therefore expected to reduce the number of trial runs commonly performed during the development of a freeze drying cycle.

5. Conclusion

The characteristics of a glass forming solution (10% w/v maltodextrin) have been observed for the first time by in situ measurements of the electrical properties of the solution, with the electrodes attached to the external surface of the freeze-drying vial. The time derivatives of all four parameters studied (F_{peak} , C_{peak} , R and C) provided a glass transition for the malto-dextrin solution in close agreement with DSC measurements ($-17\text{ }^{\circ}\text{C}$) and to a precision of $\pm 0.5\text{ }^{\circ}\text{C}$. These measurements were taken during the re-heating stage of the annealing phase, for the specific reason that these conditions more closely resemble the direction of heat flow used in DSC measurements (i.e. the registration of the glass transition on heating of a pre-frozen solution). It is also pertinent to mention that the measurement of the glass transition during the initial freezing phase would also be possible with this technique. However, this would first require a modification to the freezing process, whereby the solution is first held at a temperature close to, but below, the melting point in order to allow the ice to form and the heat to dissipate prior to re-starting the cooling process and taking the solution through the glass transition temperature.

The fact such measurements may be taken on clusters of vials, without impacting the hexagonal spatial arrangement that one normally expects to have in a production scale drier, suggests an opportunity for using such measurements in the development and monitoring of the freeze-drying cycle, in a way that could not be achieved with current techniques. It was also demonstrated that an impedance measurement with external electrodes could be used to characterise the glass forming properties of the solution. The first notable observation was that energy of activation energy below T_g was of an approximate magnitude to that of the hydrogen bond ($\Delta H \sim 20\text{ kJ mol}^{-1}$) and suggests that the charge transport mechanism under-pinning the electrical conductivity of the frozen solution was proton hopping. Finally, the fragility index ($I_f = 0.9$) of the glass forming liquid, above T_g , was calculated from the VTF function. The significance of this parameter to the freeze-concentration and stability of biopharmaceuticals, in particular, was then highlighted.

Future work

Our next articles will examine the impact of annealing on the amount of ice formation, the properties of the unfrozen fraction above and below the glass transition temperature, and the primary drying time for the product.

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