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Research paper

Through-vial impedance spectroscopy of critical events during the freezing stage of the lyophilization cycle: The example of the impact of sucrose on the crystallization of mannitol

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ABSTRACT

The aim of this work was to evaluate the application of through-vial impedance spectroscopy in the measurement of eutectic crystallization during the freezing stage of the lyophilisation cycle.

Impedance measurements of various sugar solutions (mannitol 5%, 10% and 15% w/v, sucrose 5% w/v and mannitol 5% w/v, and sucrose 5% w/v solutions) were taken during a freeze–thaw cycle, over a frequency range 10–10^6 Hz with a scan interval of 1.5 min, using measurement vials with externally attached electrodes connected to a high resolution impedance analyzer.

Estimates for the electrical resistance of the mannitol solutions record the exothermic crystallization of mannitol at a temperature of −24 °C during the temperature ramp down stage of the freezing cycle, which is in close agreement with the off-line DSC measurement of −22 °C. The freezing profile of a 5% mannitol solution with 5% sucrose (a component that does not crystallize in the frozen solution) demonstrated the inhibition of mannitol crystallization (with the implication that the product will then require sub-T_g' freezing and drying).

The work suggests a role for through-vial impedance spectroscopy in the concurrent development of the product formulation and freeze drying cycle without the uncertainty introduced when using off-line date to define the critical process parameters.

1. Introduction

The development of optimized freeze drying formulations and process cycles is based on the development of a set of customized process conditions which take into account the specific temperatures that define the physical state(s) of the formulation [1], i.e. the glass transition temperature (T_g'), eutectic temperature (T_{eu}) and the collapse temperature (T_c) [2,3]. This requires the physical characterization of the solution formulation (API and excipients) during the freezing and re-heating of the solution in order to identify these critical temperatures [4].

During freezing, a significant fraction of the water phase separates out from the solution as ice crystals while the remaining water forms a concentrated solution with the solute; this phenomenon is commonly referred as freeze concentration or cryo-concentration. The frozen matrix resembles a honey comb structure wherein the 'cells' are derived from the ice crystals while the 'walls' are formed by the viscous solution. As the product temperature is reduced further, the solute concentration approaches a critical level which favors the eutectic crystallization of solute and water molecules (The concentration at which crystallization occurs is termed eutectic crystallization C_{eu}.). The temperature associated with the onset of eutectic crystallization is recorded as the eutectic temperature or T_{eu}. Identification of this temperature is desirable in the development of a freeze drying cycle, as the crystalline matrix results in elegant cake structure [5]. Furthermore, crystallization of solutes such as mannitol and glycine enables the use of higher temperatures for primary drying than their amorphous counterpart thereby facilitating faster drying rates. As the crystalline matrix does not contain a large fraction of bound water after primary drying, as with the amorphous

Abbreviations: TVIS, through vial impedance spectroscopy; FDM, freeze drying microscopy; DSC, differential scanning calorimetry; XRD, X-ray diffraction; T_g, glass transition temperature; T_{eu}, eutectic crystallization temperature; CPE, constant phase element; *R*, resistor; *C*, capacitor.

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structures, it is less vulnerable to collapse during the secondary drying temperature ramps. The product attributes, such as the cosmetic appearance of the freeze dried cake and improved storage stability, are therefore ensured by including excipients (mannitol and glycine) which are prone to crystallize in the frozen state [1]. However, the crystallization behavior is impacted by the non-crystallizing components (e.g. sucrose) included in the formulation as well as the processing conditions, including cooling rates, freezing temperatures and/or annealing temperatures and annealing times [6]. This necessitates monitoring of the individual formulation in order to characterize the potential impact of solute crystallization.

A variety of techniques including differential scanning calorimetry, electrical resistance [7], dielectric analysis [8] freeze drying microscopy [9], Raman spectroscopy [10,11], and X-ray diffraction [6,12] have been used to identify the eutectic crystallization within frozen solutions. These are discussed in turn below:

Differential scanning calorimetry (DSC): Solute crystallization is identified as an exothermic event during the off-line thermal scanning of the formulation.

Polarized light freeze-drying microscopy (FDM): This technique has also been used to observe eutectic crystallization and melting temperatures. The anisotropicity of the crystallized sample confers a degree of optical rotation of plane polarized light according to the orientation of the solute crystals [13].

Electrical resistance: The changes in electrical impedance of the formulations, measured at fix frequency 1 kHz by means of an impedance probe placed securely in a cryostat measurement cell containing the sample (by monitoring the imaginary impedance, $Z'' = Z\ \mathrm{Sin}\Theta$) show a good correlation with thermal events such as eutectic melting as the sample is heated through the temperature range of −100 °C to 0 °C [7]. The onset of this decrease in impedance (T_{onset}) relates well to the eutectic crystallization or glass transition of the frozen matrices. However, being invasive, this technology may add further ice nucleation sites during the freezing stage [13].

X-ray diffraction (XRD): This technique evidences the crystallization of different components from their diffraction pattern as the incident X-ray beam transmits through the object (i.e. the frozen solution). The responses are displayed typically as a range of peak(s) at specific degrees in what are known as 2θ plots [6,14].

Raman spectroscopy: Crystallization in a solution is manifested as a set of peaks at discrete wavenumbers in the applied energy range (200–4000 cm^{-1}) (for example 215 cm^{-1} for ice and 1000–1170 cm^{-1} for mannitol) [10].

Dielectric spectroscopy: Monitors the relaxation of dipoles and interfacial polarizations in the frozen solution during cooling and re-heating across a broad frequency band. The peak in the imaginary permittivity, centered on discrete frequencies or temperatures, highlights the time constant for specific dipole polarizations, whereas step-changes in the real and/or imaginary permittivity, with temperature, are characteristic of phase changes such as solute crystallization [8].

Which so ever technique is used the crystallization temperatures so derived are then employed to set up the freezing conditions in the lyo-cycle. However, it should be recognized that the crystallization tendencies of a solute from solution are influenced by cooling rates which are in turn defined by the mechanisms of heat transfer for the system in question (including both contact conduction and convection). The differences in sample volume (i.e. a few microlitres in DSC/FDM vs a few mL in lyophilization) and the sample holder design and thermal mass (e.g. glass vs aluminum pans in freeze-drying and DSC, respectively) may therefore lead to the differences in the kinetics of crystallization and other thermal behavior. As a consequence the crystallization behavior of a solute may differ in the vial from that of the DSC, FDM or electrical resistance.

The measurement of solute crystallization within a glass vial has been reported in literature by using by fiber optic Raman probe [10]. The technique is advantageous in that it characterizes the event within the glass vial in a non-invasive way. However the bulky probe cannot reach the vials within the core of the usual hexagonal array employed for a fully loaded shelf. There is therefore a requirement for another analytical technique for the measurement of these critical events within glass vials which are arranged as hexagonal arrays on the freeze drier shelf, as this will then provide more realistic assessment of solute crystallization, which can also take into account the temperature distribution heterogeneities arising from spatial positions of the vials.

Our recent explorations of through-vial impedance spectroscopy as a process analytical techniques in freeze drying have shown its application in the characterization of different stages of the freeze drying process, namely, product cooling, ice formation, the glass transition and primary drying [15–17]. The technique has a distinct advantage over other single vial process analytical technologies as it does not invade the formulation (as would be the case for the thermocouple probe) nor does it perturb the heat flow (as would be the case for the microbalance approach) and allows the monitoring of vials within the usual hexagonal arrays of a fully loaded shelf.

The current study aims to evaluate the application of through-vial impedance spectroscopy in the identification of critical product parameters including ice formation and solute crystallization (eutectic formation) of solutions containing different concentrations of mannitol (5%, 10% and 15% w/v). In addition the impact of sucrose on the eutectic crystallization of mannitol was recorded during freezing.

2. Materials

Mannitol and sucrose were purchased from Sigma–Aldrich UK and used as supplied in the preparation of a number of surrogate formulations.

3. Methods

Surrogate formulations were prepared from materials having different physical characteristics, i.e. those which crystallize and those which form amorphous solids i.e. mannitol and sucrose respectively. The former was analyzed at concentration of 5%, 10% and 15% w/v while the concentration of later was gradually increased from 1% to 5% w/v in solutions of mannitol (5% w/v) in order to record the crystallization behavior. Aliquots of 3.0 ml were introduced to the impedance measurement vials ($N = 5$); The measurement vial [16] 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 × 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 measurement vials connect to the high precision impedance analyzer through coaxial cables. A HETO 08 Freeze drier; with installed the measurement system, was used for the investigation of the drying process.

Impedance measurements were taken over a frequency range 10^1–10^6 Hz with the scan interval 1.5 min. The temperature was measured using a type K thermocouple at time intervals concurrent with the impedance measurements. The freeze–thaw cycle followed the regime: Hold the shelf at 25 °C for 30 min; Temperature