OPTIMIZATION OF LYOPHILIZATION (FREEZE-DRYING) USING DIELECTRIC ANALYSIS (DEA)

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SUMMARY

Moisture content in lyophilized materials is important because it affects the long term stability of those materials. Thermal analysis techniques, in particular Differential Scanning Calorimetry and Moisture Evolution Analysis have traditionally been used to characterize the moisture-related characteristics of lyophilized materials (1,2). Dielectric Analysis (DEA), however, is another thermal analysis technique which can be used to characterize those materials. Furthermore, DEA provides better sensitivity and more flexibility for evaluating solid/liquid samples.

INTRODUCTION

In developing parenteral products, the drug candidate being formulated often exhibits poor solution stability. In those cases, the liquid formulation is usually lyophilized into a dried dosage form which has enhanced stability. Then, prior to use, the product is reconstituted with water to form the desired solution.

In lyophilization (freeze-drying), the product is initially cooled to low temperatures (generally -40°C or lower) where it becomes frozen or solidified. Then, primary drying is initiated by applying a modest vacuum and warming the freeze-dryer shelves to remove the bulk water present through sublimation. Once the majority of the bulk water has been sublimed, secondary drying is initiated to remove sorbed or residual water from the product by further increasing the shelf temperature and reducing the chamber pressure.

During the primary drying process, the rate of sublimation can be accelerated by increasing the temperature of the product. Since the lyophilization time, and hence the cost associated with the lyophilization process, is mainly influenced by the primary drying process, processors would like to perform this step at the highest temperature possible which still retains product integrity. This temperature which is called the maximum allowable product temperature (3) is dependent on the physical state (crystalline, amorphous glass) of the frozen material. Three different physical states (situations) are usually encountered with real-world solutions, and each of these must be treated differently in determining the maximum temperature for lyophilization. Those situations are summarized below:

1. The solution only crystallizes or forms a eutectic when cooled. The maximum allowable product temperature in that situation is the melting point or eutectic temperature respectively.
2. The solution crystallizes during slow freezing, but forms an amorphous glass with water during rapid freezing. The maximum allowable product temperature in that situation is the temperature of crystallization (change from glassy to crystalline structure during heating), unless that transition occurs via annealing at a temperature below the onset of any sublimation (3).
3. The solution forms an amorphous (glassy) structure when cooled and does not crystallize upon freezing or through annealing. The glass transition temperature is the maximum allowable product temperature in that situation.

Historically, processors of lyophilized materials have used electrical resistivity (ER) and freeze drying microscopy to determine the maximum allowable product temperature. Thermal analysis techniques which measure changes in the physical properties of materials provide an alternative to those traditional techniques. Specifically, differential scanning calorimetry (DSC) and dielectric analysis (DEA) are the thermal analysis techniques best suited for this measurement. Furthermore, dielectric analysis (DEA) is the best suited technique because it has sufficient sensitivity to determine the maximum allowable product temperature in all three of the situations described earlier. This study illustrates typical DEA results for each of the three product situations.

EXPERIMENTAL

Dielectric analysis is an analytical technique which measures the two fundamental electrical characteristics of a material - capacitance and conductance - as a function of time, temperature, and frequency. The capacitive nature of a material is its ability to store electric charge, and the conductive nature is its ability to transfer electric charge. While these electrical properties are important in themselves, they have even more significance when correlated to molecular activity. Such correlations allow key transitions in the material including melting, glass transitions, and even weaker secondary molecular transitions to be followed. It is this ability to detect molecular transitions which is primarily of interest when evaluating lyophilized materials.
The dielectric measurements described in this paper were performed with the TA Instruments DEA 2970 Dielectric Analyzer. Several sensor configurations are available with that instrument to accommodate a wide variety of sample types. The ceramic single surface sensor (Figure 1) was used here because it readily allowed initial application of the liquid material at room temperature, yet remained properly aligned and calibrated as the sample changed to solid form for measurement. Furthermore, a related remote single surface sensor is available which would allow these measurements to be made inside the actual lyophilization unit if desired. The actual experimental conditions used were:

- 1ml of sample solution
- 1-10°C/minute cooling rate to initial temperature
- 1°C/minute heating rate during measurement
- multiple measurement frequencies in the range 0.1-1000Hz
- 500 ml/minute dry nitrogen purge

Plots of $\varepsilon'$ (permittivity) and $\varepsilon''$ (loss factor) were chosen to track behavior.

**RESULTS**

A 10% w/w sodium chloride solution was chosen to evaluate the dielectric behavior for a simple eutectic system. Figure 2 shows the results. Two transitions at -21 and -6°C are observed. The frequency independence of those two transitions (i.e. the temperature at which the transitions occurs does not shift with differences in frequency) indicates both are first order transitions. Comparison to literature values readily confirms the transitions as melting of a sodium chloride/ice eutectic and the depressed melting of ice respectively. Figure 3 shows the partial phase diagram constructed based on the DEA results for a range of sodium chloride/water compositions. DSC results are also shown in Figure 3 to indicate the excellent agreement between the two techniques for crystalline/eutectic systems.

A 5% w/w solution of D-Mannitol was selected to evaluate dielectric behavior for a solution whose crystalline/amorphous nature is governed by initial cooling rate and annealing. The log $(d\varepsilon'/dt)$ versus temperature plot after rapid cooling at 10°C/minute indicates three transitions (Figure 4). The two higher temperature transitions at -5 and -1.8°C represent eutectic melting and bulk water melting respectively. The third transition at -27.5°C is frequency independent indicating that transition is also a first order transition. Further evaluation verified that the transition is due to an amorphous glass to crystalline conversion. Since its temperature is above the onset of sublimation, that transition is the maximum allowable product temperature for that solution. The DSC results (Figure 5) show similar transitions.
Figure 2: 10% SODIUM CHLORIDE SOLUTION
DEA RESULTS

![Graph showing DEA results of 10% sodium chloride solution with single surface sensor sensitivity and NaCl/Water eutectic melt temperature.]

Figure 3: PARTIAL PHASE DIAGRAM
NAACL / WATER SOLUTION

![Graph showing partial phase diagram for NaCl/water solution with DSC onset values, DEA peak values, and literature values.]
Figure 4: D-MANNITOL EVALUATION BY DEA

Single Surface Sensor
0.1, 0.5, 1, 5, & 10Hz

5% D-Mannitol Cooled Rapidly

Amorphous to Crystalline Transition ca. -27.7°C

Temperature (°C)

Heat Flow (W/g)

Figure 5: D-MANNITOL EVALUATION BY DSC

5% D-Mannitol In Water Cooled At 10°C/minute Before Evaluation

Amorphous to Crystalline Transition Onset ca. -26.5°C

Combined Eutectic Melt & Depressed Melting of Ice ca. -2.3°C
A 10% w/w sucrose solution was chosen to represent amorphous (glassy) behavior. After cooling at 1°C/minute, the DEA results show a high temperature, frequency independent transition (Figure 6) which corresponds to the depressed melting of ice. Several lower temperature transitions are also observed (Figure 7). The smaller magnitude and frequency dependence suggest that these transitions are second order (or higher) transitions associated with molecular motions in the material. The higher temperature of those transitions is likely to be the glass transition. However, because of its broad frequency dependence and magnitude it is difficult to assign a specific glass transition temperature corresponding to the maximum safe product temperature.
A novel approach called the “Take-Off Frequency Method” resolves this problem. The “take off frequency” (TOF) refers to the lowest frequency in a Debye plot (Figure 8) where dipole alignment can be achieved without being obscured by DC conductivity such that:

$$\text{TOF (T)} = \text{TOF}_0 e^{(H/RT)}$$

where T is temperature in K and H corresponds to the slope of the ln (TOF) versus 1/T plot.

The TOF values are obtained by fitting the $\varepsilon''$ - frequency data (dipolar) corresponding to the particular temperature isotherm to a best fit function and then determining the value of TOF for that function. The TOF plot (Figure 9) for the sucrose solution reveals two distinctly linear regions with the intersection corresponding to the $T_g$ or the maximum safe product temperature. The temperature value predicted using the TOF method is highly consistent with respect to the literature value (-32°C). In this solution, unlike the two previous situations, DSC does not have sufficient sensitivity to detect the $T_g$ - maximum product temperature.
REFERENCES

2. TA Instruments Applications Brief Number TA-114.