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Research Article

Freeze-Dry Microscopy: Impact of Nucleation Temperature and Excipient Concentration on Collapse Temperature Data

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Abstract. The objective of this study was to investigate the impact of nucleation temperature (T_n) and excipient concentration on the collapse temperature data obtained from freeze-dry microscopy (FDM) experiments. T_n, the temperature of the onset of collapse (T_{oc}), and the full collapse temperature (T_{fc}) were determined for aqueous solutions of polyvinylpyrrolidone (PVP) 40 kDa and 2-(hydroxypropyl)-ß-cyclodextrin. Concentrations were varied from 1% to 20% (*w*/*w*) for PVP and from 1% to 30% (*w*/*w*) for the 2-(hydroxypropyl)-ß-cyclodextrin. Mutual correlation coefficients were calculated for the observed T_n, T_{oc}, and concentrations of the solutions. In addition, outliers were detected and eliminated by applying the leaving-one-out routine and calculating correlation coefficients without it. T_n was found to be non-correlated with concentrations and only weakly correlated with T_{oc}. The correlation between these two temperatures was particularly poor for the solutions of the highest and lowest concentrations. In contrast, T_{oc} correlated much better with the corresponding concentrations, resulting in a quadratic fit for PVP and a linear fit for 2-(hydroxypropyl)-ß-cyclodextrin.

KEY WORDS: collapse temperature; concentration; freeze-dry microscopy; nucleation.

INTRODUCTION

Freeze-drying is known as an important and frequently used drying technology for biopharmaceutical products which are not stable in aqueous solution over an acceptable shelf life (1). Biopharmaceuticals are often used to fight cancer, cardiovascular or diabetic diseases, or viral infections. To increase production turnover while assuring a high quality of such costly medication, the critical process parameters of the freeze-drying cycle must be optimized (2). Optimization of a freeze-drying cycle means to reduce cycle time. This can be achieved by optimizing in particular primary drying conditions by selecting appropriate chamber pressure and shelf temperature settings. As a general rule, the product temperature at the ice sublimation interface must be maintained just below the critical formulation temperature throughout this process step (3). The critical formulation temperature can be either determined by freeze-dry microscopy (FDM) or differential scanning calorimetry (DSC) and is denoted collapse temperature (T_c) for the FDM or glass transition temperature of the maximally freeze concentrated solute (T_g') for DSC experiments. However, it has been reported in the literature that the measure of T_g' is not necessarily representative for the sublimation procedure in a vial during a freeze-drying run (4). Therefore, freeze-dry microscopy has become the method of choice for critical temperature measurements over the last couple of years (5). Collapse in a given region of the product results from surface tension induced viscous flow of the amorphous phase after the ice–vapor interface has moved past that particular region (4). When performing primary drying above T_c, one may observe loss of structure (denoted as "shrinkage" or "collapse") in the dried region adjacent to the ice–vapor interface due to a glass transition in the amorphous product (6). Shrinkage or collapse may compromise the (predefined) product quality attributes, e.g., product stability, residual moisture content of the cake, reconstitution times, etc. (7).

Recent studies focused on a rational design of FDM methodology, based on various collapse temperature measurements and different model systems (8). For a representative T_c measurement and subsequent optimization of the freeze-drying process, the collapse detection must be as exact and representative as possible and the influencing parameters must be known and taken into consideration during the experiment.

When applying the cooling step during an FDM experiment, nucleation of the sample can neither be controlled nor predicted. As a result, differences in the size of ice crystals among samples may be observed in the course of experimenting, even under identical experimental conditions. This would also be expected during a conventional freeze-drying run and has already been discussed excessively in the literature: the higher the supercooling of the solution, the less time remains for ice crystal growth and the smaller is their size. In 2001, Searles *et al.*

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published a study which confirmed that the stochastic nature of nucleation led to heterogeneity among samples concerning the freezing mechanism, morphology and primary drying rate (9). Out of this reason and coincidental factors (particles, damaged glass cover slides), replicates of FDM measurements are unlikely to ever produce exactly the same frozen structure. When the formation of ice crystals is different between replicate runs in an FDM experiment, the formation of different pore sizes during the ice sublimation phase is expected to have an influence on resistance of the dried structure to vapor flow which, in turn, might influence drying rate and collapse behavior (4,9–10). Apart from the pore size distribution of the dried matrix, collapse behavior depends on the total solid content of the excipient (4).

In the present study, mutual correlation between the nucleation temperature (T_n), the temperature of the onset of collapse (T_{oc}), and the total solid content of the excipient was for the first time evaluated by FDM experiments. Based on the excessive number of data points and the use of two different model excipients, it was possible to conduct such a detailed investigation of interrelation between T_n, T_{oc}, and concentration.

MATERIAL AND METHODS

Two excipients commonly used in freeze-drying formulations were selected as model substances: polyvinylpyrrolidone (40 kDa), a polymer used as a cryo- and lyoprotectant, and 2-(hydroxypropyl)-ß-cyclodextrin, a commonly used collapse temperature modifier. Polyvinylpyrrolidone (further denoted as PVP) and 2-(hydroxypropyl)-ß-cyclodextrin (further denoted as HP-ß-CD) were of highest analytical grade (Fluka, Switzerland) and used as received. Aqueous solutions were prepared as stock solutions with double distilled water from an all-glass apparatus (Destamat Bi 18 T, Heraeus, Hanau, Germany) and aliquots filtered through a 0.22-µm membrane (Millipore®, Billerica, MA, USA) prior to use. Total solid contents ranged from 10 to 200 mg/g (1–20% *w*/*w*) for PVP and from 10 to 300 mg/g (1–30% *w*/*w*) for HP-ß-CD.

Freeze-Dry Microscopy

All solutions were studied by performing up to five FDM measurements (n=5). The freeze-dry microscope consisted of a microscope (Axio Imager.A1, Zeiss, Germany) with a lambda plate plus analyzer and a FDCS 196 freeze-drying stage (Linkam, Surrey, UK). The used magnification was 200-fold. Calibration of the thermocouple in the FDM stage was performed with 10% (*w*/*w*) solutions of KCl (−10.7°C), NaCl (−21.1°C), and $MgCl_2$ (−33.6°C) by detecting the known melting points of the frozen solutions (4). Custom-made, precision-cut spacers (height 0.025 mm) were used to maintain a constant thickness of the sample layer. The preparation of the samples was as follows: a 15-mm round glass cover slide was placed on the silver block of the freezing stage. This block was coated with a 5-µL drop of silicon oil to improve heat transfer from the silver block to the glass cover slide. Then, about 2 µL of solution were pipetted onto the 15-mm glass and subsequently covered by a second 9-mm cover glass slide, using the spacers in-between both slides.

The cooling rate used throughout this study was 10°C/min. The cooling rate in this context represents the preprogrammed ramp rate used in the FDM software (Linksys 32, Linkam, Surrey, UK). Note that the rational behind the use of a 10°C/min cooling rate in this study is to simply make the experimental time more appropriate for the amount of data required for statistical analysis. Further, a much slower cooling rate was not expected to show a significant difference in the results obtained, based on a recent report which showed for 5% (*w*/*w*) aqueous solutions of sucrose no difference in collapse temperature measured by FDM when using a cooling rate of 1°C/min and of 10°C/min (11). These results might be perfectly plausible if the variability of the nucleation temperature during an FDM experiment is taken into consideration, even when using the same sample and identical experimental conditions (i.e., T_n data were found exactly in the same temperature range for a 1°C/min and 10°C/min cooling rate) (12).

The heating rate was 1.0°C/min in the temperature range of interest to allow a representative measure of the first structural changes in the product. The pressure in the stage (P_s) was measured using a calibrated Pirani gauge and was controlled by a motor valve below 0.03 mbar (22.5 mTorr). Pictures of the sample were taken in 1-s intervals by using a digital camera system which was mounted on top of the microscope. Pictures were then analyzed for the nucleation (T_n), onset of collapse (T_{oc}), and full collapse temperature (T_{fc}), using the Linksys 32 software. The nucleation temperature T_n was determined using the first recorded picture illustrating a frozen structure of the measured solute. The T_{oc} was defined as the temperature at which first gaps and fissures were visible adjacent to the sublimation interface. Full collapse (T_{fc}) was defined as the temperature at which a full loss of structure (i.e., no connection between dried product matrix and sublimation interface) could be determined.

Statistical Data Analysis

The interrelation between the critical parameters of the formulations was assessed via correlation coefficient calculations using MATLAB® (MathWorks, Natick, MA, USA) which was also used for plotting all figures. In addition, driven by the issues spotted in preliminary analysis of the experimental data, a validation routine known as leave-one-out (L-O-O) has been applied. The L-O-O concept is often used in multivariate analysis, e.g., in multivariate calibration studies with near-infrared spectra from various materials (13). In short, in the L-O-O approach one of the samples is left out from the rest of the samples that are used to build a calibration model which is then used for prediction of a variable of interest (e.g., concentration) in future samples. The effectiveness of the obtained model is validated by predicting the concentration of the sample that was left out, thus L-O-O can be considered a validation approach. This operation is then repeated n times, with n being the number of samples and during each cycle, another sample is left out and used for validation. This is a popular way to validate a calibration model on a rather limited number of samples. All L-O-O calculations in this study were performed in MATLAB® without any special routine being created. More specific reasons for introducing the L-O-O approach into the present analysis are given in the text below. The reader is referred to the literature for more in-depth details about the L-O-O routine (13).

RESULTS AND DISCUSSION

Interrelation between T_{oc} and T_{fc}: PVP 40 kDa and HP-ß-CD

It is common practice to report the collapse temperature for a given system as the "onset" temperature, i.e., the temperature where the first structural changes are visually detected (Fig. 1a). If the sample temperature is increased further beyond the T_{oc} boundary, the dried area loses its structure increasingly up until a temperature where no coherent sample matrix forms adjacent to the sublimation interface (Fig. 1b). The temperature interval between T_{oc} and T_{fc} is becoming of increasing interest in investigations of the temperature tolerance (i.e., the robustness of the formulation) during the primary drying phase of a freeze-drying cycle. Clearly, a great difference between T_{oc} and T_{fc} may be translated into a higher temperature tolerance of the product matrix. For example, Fonseca and coworkers reported in 2004 that a lactic acid bacterial suspensions showed a surprisingly strong bias between T_g' and T_c (up to 10°C) which implied a significant "robustness" of the formulation to temperature during the freeze-drying process (11). A statistical analysis for both PVP 40 kDa and HP-ß-CD showed that T_{oc} and T_{fc} are highly correlated for both datasets, with correlation coefficients of 0.91 for PVP and 0.98 for HP-ß-CD. For HP-ß-CD, T_{oc} ranges between −10.4°C and −6.7°C and T_{fc} between −9.1°C and −5.8°C. T_{oc} for PVP was found between −25.2°C and −17.8°C and T_{fc} between −23.9°C and −15.4°C. The results clearly illustrate that irrespective of the total solid content and T_n of the solutions, the two collapse temperatures vary very similarly. This observation is important since it implies that the temperature interval between T_{oc} and T_{fc} is based on *material properties* rather than the applied measurement methodology. Based on the investigated similarity between T_{oc} and T_{fc}, there was no need for any further involvement of T_{fc}; hence, it is not addressed in the following text.

Interrelation between T_n, T_{oc}, and Total Solid Content: PVP 40-kDa Solutions

Correlations with T_n involved were found nowhere near as simple as the correlation between T_{oc} and T_{fc}. Figure 2 and Table I illustrate the essence of the problem with regard to the correlation between T_n and T_{oc} for the PVP 40 kDa data. The experimental data appear to be broadly spread, and it is difficult to construe any correlation that may exist in this dataset simply because of the large variability of the data points. The standard deviation of T_n for all the concentrations is significant, reaching, in the worst case, even 30% for the 10% concentration (four repeats, Table I). Such a significant variability might be expected in the first instance. This observation of variability is caused by spontaneous nucleation of the solution which, in turn, can be *controlled*, neither during an FDM experiment nor during the freezing step in a freeze-drying cycle (14). Consequently, this will cause significant (inherent) variations in T_n (4). There are literature references available which report on procedures that can enhance homogeneity of the nucleation within a given batch in a freeze-dryer (14–16). However, these methods are mostly limited to laboratory scale freeze-dryers (14) or simply inapplicable for commercial scale freeze-drying due to the issues of sterility and adulteration of the compound, e.g., adding a nucleating agent like silver iodide to the formulation (16). Because of design and technical limitations neither of these methods can be applied to freeze-dry microscopy where a 2-µL volume of solution is frozen and examined.

In the present set of experiments with PVP 40 kDa, T_n data were found to vary between −22°C and −12°C. This was expected, based on reports of nucleation temperatures measured in vials in a freeze-dryer (14–16). Despite the noisy appearance of the data illustrated in Fig. 2, the distribution of the points must be more closely inspected because of the possibility that there are outliers or systematic (but explicable) variations the removal of which may result in emergence of a correlation pattern or, at least, some sort of trend. Since there were 32 samples in the PVP study, L-O-O correlation coefficient calculation was repeated 32 times with 31 samples in each cycle. The idea behind this procedure is rather simple: if there is a sample with a T_n/T_{oc} value which is perceivably different from the values for the rest of the samples, its omission should lead to increase (in the absolute values) of the correlation coefficient between T_n and T_{oc}. The correlation coefficient for all the data displayed in Fig. 2 is −0.07 which confirms the visual impression of the lack of mutual dependence between T_n and T_{oc}. However, when the three points which appear to be furthest away from the bulk of the data (marked in brackets) are excluded, the correlation coefficients significantly increase to −0.37. Correlation coef-

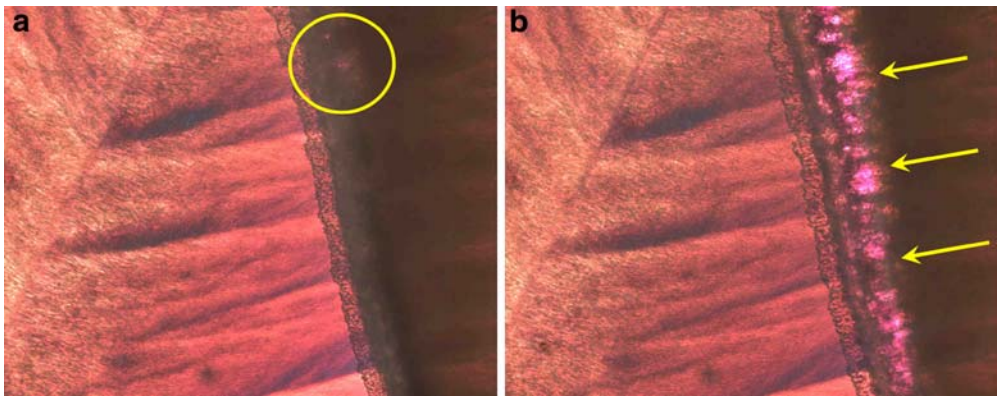

Fig. 1. Freeze-dry microscopy images of a 12% (*w*/*w*) PVP 40-kDa solution: **a** (*left*) illustrates the onset of collapse (T_{oc}) at −19.7°C (P_s=0.011 mbar), **b** (*right*) the full collapse of the same sample determined at −19.0°C (P_s=0.010 mbar)

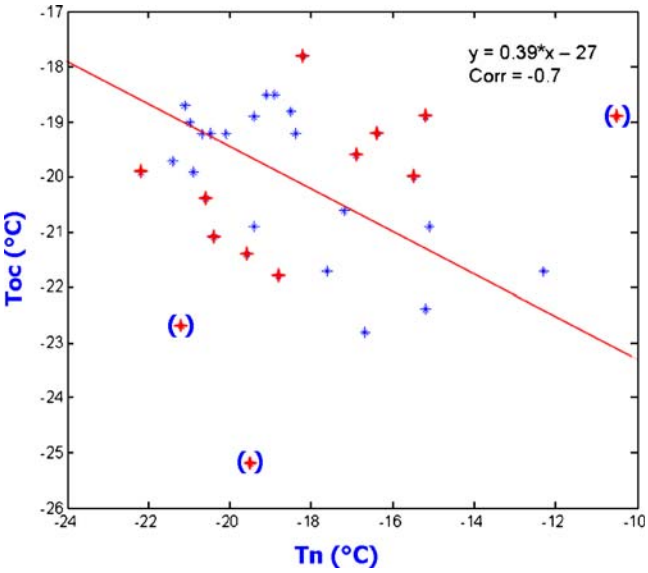

Fig. 2. Set of T_n and T_{oc} data for the PVP 40-kDa measurement series at different total solid content. All data points which were identified as outliers in the L-O-O analysis are marked with a *bold cross*. The linear fit is shown for guiding the eye; its correlation coefficient is −0.7

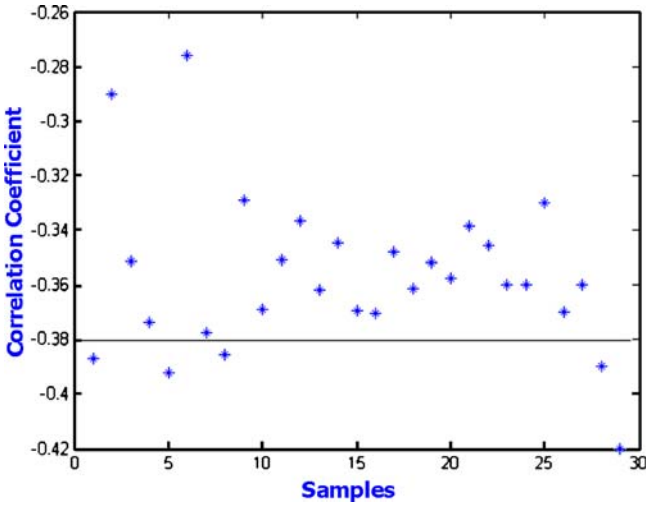

Fig. 3. PVP 40 kDa: variation of the correlation coefficients in the leave-one-out procedure after the removal of three samples from the first iteration. The *line* marks the threshold below which the samples are considered removable. They all belong to either low or highly concentrated solutions

ficients vary between −1 and +1 with +1 denoting perfectly synchronized increase or decrease of two variables and −1 denoting perfectly opposite variation, i.e., increase of one of the variables is followed by decrease of the other. Repetition of the L-O-O correlation coefficient routine with the remaining 29 samples led to further removal of samples increasing thereby the correlation coefficient to −0.58 (five samples removed). The variation of correlation coefficients during this L-O-O procedure is shown in Fig. 3. The third and final L-O-O iteration led to a T_n *vs.* T_{oc} correlation coefficient of −0.7 (five more samples removed). Figure 2 shows the final T_n *vs.* T_{oc} dependence with the total of 19 remaining samples in the dataset. The final calculated correlation coefficient of −0.7 depicts a trend, rather than a well-defined correlation. Fitting of the remaining points was performed with linear regression analysis rather than using a nonlinear approach. However, from the data illustrated in Fig. 2, a quadratic fit might also be appropriate.

Table I. T_n Data for the PVP 40-kDa Solutions, Illustrated with Increasing Total Solid Content

Concentration (%, *w*/*w*)	T_n (average; °C)	SD, T_n	Rel SD, T_n (%)
1	−16.3	1.0	6
2	−18.3	3.5	19
3	−19.1	2.3	12
5	−17.3	2.7	15
10	−17.6	5.2	30
12	−19.8	1.3	6
15	−20.0	1.2	6
20	−18.0	1.9	11

Values represent average T_n data and corresponding standard (SD) as well as relative standard deviations (Rel SD)

The outliers removed by L-O-O analysis were, in most cases, solutions of either low or high concentration. This is valuable information for the FDM measurement methodology. Formulations with low total solid content form small holes which can even be observed in the dried noncollapsed structure at temperatures much lower than T_{oc}. As a result, reliable determination and interpretation of T_{oc} data become much more difficult and are dependent on the experience of the user. As for the higher concentrations, the accurate determination of the T_{oc} is difficult as well, based on (1) the relatively low amount of light provided by the light source of the microscope, (2) the high density of the dried structure, and, thus, 3) the limited transmission of light through the sample which then allows less precise observation of structural changes. In particular for PVP 40 kDa (i.e., a polymer), the observed remaining lyophilized structure is very dense compared to other excipients used in freeze-drying. As a result, T_{oc} measurements of solutions of broadly varying concentrations are prone to show variability and are subjective to a significant degree, i.e., dependent on the individual assessment of a user performing the experiment. Consequently, a rational removal of some points from those measurements may be considered as justified.

In the next phase of the PVP study, T_{oc} was correlated to the corresponding total solid content (Fig. 4a). This correlation is much more straightforward since only a couple of points were identified to be out of the main trend (with all the measurements of the 3% sample again being outliers). Those points are ignored in Fig. 4a; thus, a distinct quadratic dependence of T_{oc} *vs.* total solid content is obtained. It is important to underline at this point that the T_{oc} *vs.* total solid content dependency was found to be clearly different from the correlation between T_n and T_{oc}. The T_{oc} *vs.* total solid content correlation plot illustrates a much stronger impact of concentration on T_{oc} which applies even for small variations in concentration. Since only a weak correspondence is found between T_n and T_{oc}, and assuming that T_{oc} is clearly

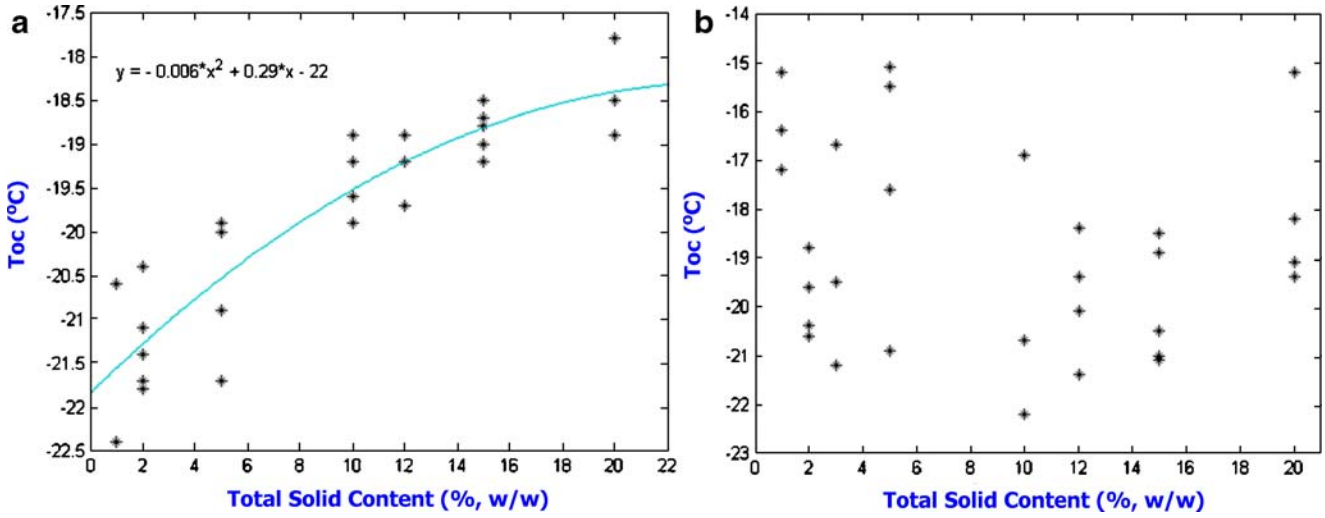

Fig. 4. PVP 40 kDa: **a** (*left*) represents an exponential fit for the variation of T_{oc} with total solid content for PVP solutions, **b** (*right*) represents the variation of T_n with the total solid content for the PVP solutions. The correlation coefficient for these points is close to 0

correlated with the total solid content, one may expect that there would be only a weak correlation between T_n and total solid content. An additional support for this assumption may be found in the work of Miyata *et al.* (17) who reported that solutions of disaccharides showed highly comparable supercooling behavior over the entire concentration range analyzed. The results of the present study largely confirm these expectations: T_n *vs.* total solid contents plot shows that T_n does not depend on the concentration (Fig. 4b).

In summary, the correlation analysis of the PVP coupled with the assessment of the quality of the data via use of L-O-O routine shows that: (1) T_{oc} increases with an increase of the total solid content and reaches a plateau for highly concentrated (i.e., >20%) solutions, the points being best fitted with a quadratic model (Fig. 4a). (2) Only a weak and negative correlation is observed for T_{oc} and T_n after excessive data treatment that eliminated significant number of the data points, most of them referring to extreme concentrations (<3% and >10%). T_{oc} measurements of those samples are believed to be prone to errors due to the inherent weaknesses in the concept of the measurement, and (3) T_n is found to be independent of the total solid content of the freeze-dried solution. Additionally, a simple multivariate analysis of the PVP dataset was performed which entirely supported the univariate analysis described above. Multivariate analysis in this context means that T_{oc} is simultaneously fitted with both concentrations and the parameter T_n. It is shown that T_n is practically independent of T_{oc} and as such it does not contribute to the characterization of T_{oc}. On the other hand, the concentrations are found to be correlated with T_{oc}. Figure 5 illustrates a 3-D representation of T_{oc}, total solid

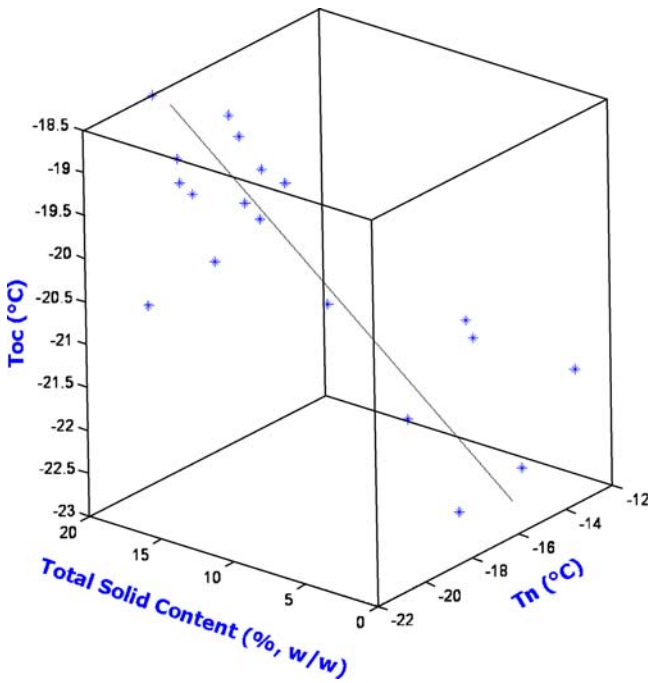

Fig. 5. PVP 40 kDa: 3D plot of T_n, T_{oc}, and total solid content. Most of the experimental points are in the *plane* spanned by T_{oc} and total solid content with T_n axis being orthogonal to that particular plane

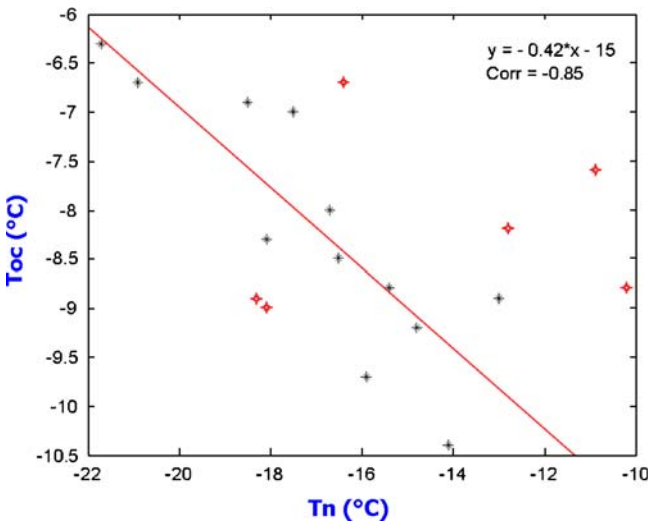

Fig. 6. HP-ß-CD: the set of T_{oc} over T_n data for the series of solutions of various total solid content. The original correlation coefficient is −0.48, the one after L-O-O analysis is −0.85 (linear fit). Outliers are marked with a *bold cross*

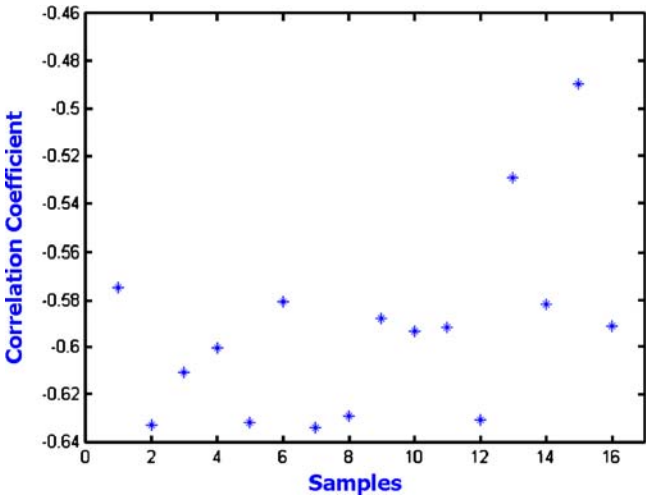

Fig. 7. HP-ß-CD: variation of the correlation coefficients in the leave-one-out procedure after one of the samples from Fig. 6 with 1% concentration is left out. On the basis of this figure, five more samples are eliminated from the original dataset

content, and T_n. Here, most of the experimental points are in the plane spanned by T_{oc} and total solid content with T_n axis being orthogonal to that plane.

Interrelation between T_n, T_{oc}, and Total Solid Content: HP-ß-CD

The original data points for relation for 2-(hydroxypropyl-)ß-cyclodextrin (HP-ß-CD) are shown in Fig. 6. A negative trend is more perceivable for these data and, again, there are a few points that appear to be far from the bulk of the data (Fig. 6, right-hand side). At the first glance, these data appear to be more coherent in comparison to those in Fig. 2, and this is also reflected in the correlation coefficient of −0.48 with all the experimental points considered. Similarly to the previous case, the first cycle of L-O-O correlation coefficient calculations immediately identifies one of the samples with concentration of 1% as an outlier (not plotted). The correlation improves to −0.59 after the elimination of that particular sample. The second iteration of the L-O-O correlation coefficient analysis, however, reveals five more samples which seem to spoil the trend (Fig. 7). This analysis appears to be more convincing than that with the PVP solutions because of the clearer distinction of the five identified samples in Fig. 7. With their elimination (indicated with a bold cross in Fig. 6), the remaining total of 12 samples shows an obviously negative correlation that again may be fitted by either a linear or a quadratic function. A linear fit is chosen in order to be more consistent with the employed correlation terminology that better corresponds to linear dependencies. The dispersion of the points of the low-concentration samples (that is the highest T_n and the lowest T_{oc}) prevents the nature of dependence to be more clearly determined, i.e., both fits are similarly accurate.

As mentioned above, the uncertainties in the measurement technique are believed to be the major cause of the dispersion of the data. For this set of solutions, five out of the six removed points deviate quite significantly from the samples with similar concentrations, or replicate measurements for the same concentration show a high variance. For example, the three T_n measurements for a 1% (*w*/*w*) concentration of HP-ß-CD vary from −11°C to −18°C which spans almost the entire range of T_n values for all the evaluated concentrations. The removed data points that were obtained from the 10% solutions have an unreasonably high T_n that places the corresponding T_{oc} data points to the far right-hand side in the plot. This may even appear to be a systematic error for that particular sample. Finally, T_n values for the 20% solutions vary between −16°C and −21°C which is a significant relative deviation, keeping in mind that the average value would be −18.5°C. The results obtained for HP-ß-CD, hence, strengthen the observation made for the PVP data: the measurements of solutions with low and high concentrations seem to be rather unreliable or irreproducible so that it is difficult to ascertain exact correlation between T_{oc} and T_n. For the HP-ß-CD data, total of four outliers belong to those boundary concentrations (two from 1% and two from 20%).

Figure 8a, b illustrates correlations between T_{oc} and T_n *vs.* total solid content, respectively. These two plots essentially

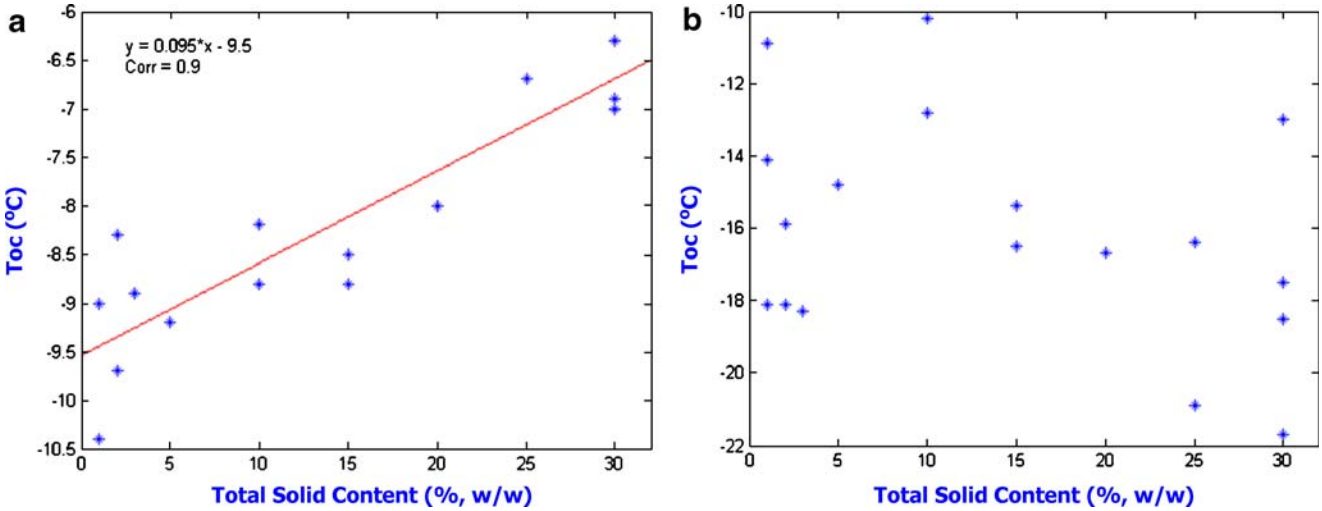

Fig. 8. HP-ß-CD: **a** (*left*) shows variations of T_{oc} over total solid content for HP-ß-CD solutions and reveals a strong linear correlation, **b** (*right*) illustrates T_n *vs.* total solid content for HP-ß-CD solutions where the correlation coefficient for these points is close to 0

concur with the corresponding correlations for the PVP data (Fig. 4a, b). The correlation between T_{oc} and the corresponding concentrations is unquestionable, although in this particular case, it appears to be more linear than quadratic (two obviously outlying points were excluded to produce Fig. 6). T_n again appears to be independent of total solid content (Figs. 4a, 8a).

To summarize the results for the HP-ß-CD experiments, T_n clearly exerts more impact on T_{oc} than for the PVP 40-kDa solutions but still has a marginal effect when compared with the effect of variation in concentration on T_{oc}. PVP is a mixture of molecules with different chain lengths while HP-ß-CD has a defined chemical structure. Hence, for PVP higher variations in T_{oc} (and T_{fc}) were expected reflecting more variations in the composition of the frozen structure observed. Because of these higher variations in T_{oc} for PVP 40 kDa, T_n has a higher impact on T_{oc} for HP-ß-CD.

CONCLUSION

The results obtained during this study suggest that T_n has an influence on the frozen structure and consequently on the corresponding T_{oc} and T_{fc}. Since T_n cannot be controlled during FDM measurements, a user should consider (at least) three to five individual measurement of a single solution (resulting in different T_n), and only the mean value out of those measurements should be taken into account for further cycle development and optimization. Significant deviations in T_n are probably unavoidable. Furthermore, low (<3% *w*/*w*) and high (>10% *w*/*w* for PVP 40 kDa, >20% *w*/*w* for 2-(hydroxypropyl-)ß-cyclodextrin) total solid contents are found to be less reproducible and much more prone to outliers. For solutions with a low total solid content, the holes in the dried structure perturb the possibility for accurate detection, while for highly concentrated solutions, the high density of the dried area causes observation problems of the collapse. These two effects are essentially thought to be behind the outliers of T_n *vs*. T_{oc} correlations. Finally, there is a clear correlation between T_{oc} and the corresponding concentrations. For PVP 40-kDa solutions, quadratic relation of T_{oc} over concentration is found, whereas a linear correlation is determined for HP-ß-CD. These dependences may be useful for further optimizations of formulations by varying the contents of the excipients. The existing data are rather well fitted by both linear and nonlinear functions, so it remains unclear (at this point) which of these two is recommendable.

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