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Thermal expansion measurements of cryoprotective agents. Part II: measurements of DP6 and VS55, and comparison with DMSO☆

Yoed Rabin* and Ernest Bell

Department of Mechanical Engineering, Carnegie Mellon University, Pittsburgh, PA 15213, USA

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Abstract

As part of an ongoing effort to characterize the mechanical behavior of biological tissues in the cryogenic temperature range, the current study focuses on thermal expansion measurements of cryoprotective agents. Utilizing the experimental apparatus described in the previous report (Part I), the current report (Part II) includes thermal expansion measurements of the cryoprotectant mixtures DP6 and VS55, and comparison with available data from the literature on DMSO. In the temperature range in which the cryoprotectant mixture behaves like low viscosity liquid, results of this study show that the thermal expansion coefficient of VS55 and DP6 is 22% and 40% lower than that of 3 M DMSO, respectively, where 3 M DMSO is only one component of each cryoprotectant mixture. This significant difference is attributed to the presence of 3 M formamide in VS55.

Keywords: Cryopreservation; Cryoprotectant; Solid mechanics; Thermal expansion; DP6; VS55; DMSO

The volume change associated with change in temperature of the material is known as 'thermal expansion.' The ratio of the thermal expansion to the initial volume is known as 'volumetric thermal strain.' The term 'linear thermal strain' is defined as one third of the volumetric thermal strain. The term 'linear thermal expansion coefficient' represents a physical property indicating the rate at which the linear thermal strain changes with temperature. A positive value of the linear thermal expansion coefficient indicates an increase in volume with the increase in temperature (volume expansion), while a negative value indicates a decrease in volume with the increase in temperature (volume contraction). In the context of this report, the term 'thermal expansion' is used as a generic term indicating the physical process, irrespective of the sign of the linear thermal expansion coefficient. Unless otherwise specified, the terms 'linear thermal strain' and 'thermal strain' have the same meaning in this report. Likewise, the terms 'linear thermal expansion coefficient' and 'thermal expansion coefficient' have the same meaning.

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* Corresponding author. Fax: 1-412-268-3348.
E-mail address: rabin@cmu.edu (Y. Rabin).

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As a part of an ongoing effort to characterize the mechanical behavior of biological tissues in the cryogenic temperature range [3–9], the current study focuses on thermal expansion measurements of cryoprotectants. The current study focuses on the upper part of the cryogenic temperature range, where the cryoprotectant can be considered to be low viscosity liquid at all practical cooling rates, whether during vitrification or during classical cryopreservation. In the solid mechanics sense, liquid can be defined as a material which continues to deform under the application of a constant load; low viscosity indicates that the deformation rate is relatively high for low loads. The current study also addresses the effect of volume change associated with crystallization.

The experimental apparatus used in this study has been described in detail in the previous report (Part I). The current report focuses on thermal expansion measurements of the cryoprotectant cocktails DP6 and VS55, which are of current interest in cryopreservation research and application [13]. The current report also includes comparison with available data from the literature on DMSO, which is one the most commonly used cryoprotectants in classical cryopreservation, and which is also a dominant ingredient in the mixtures DP6 and VS55.

Materials and methods

Two cryoprotectant solutions have been experimented upon in this study: VS55 and DP6 (Organ Recovery Systems). VS55 is a mixture of: 242.14 g/L DMSO (3.1 M), 168.38 g/L propylene glycol (2.2 M), 139.56 g/L formamide (3.1 M), 2.4 g/L Hepes, in EuroCollins solution. The EuroCollins solution is a mixture of: 34.95 g/L dextrose, 7.3 g/L K_2HPO_4, 2.04 g/L KH_2PO_4, 1.12 g/L KCl, 0.84 g/L $NaHCO_3$. DP6 is similar to VS55 with the exclusion of formamide: 234.4 g/L DMSO (3 M), 228.3 g/L propylene glycol (3 M), 2.4 g/L Hepes, in EuroCollins solution. VS55 is a well-established cryoprotectant [2,11,12], while DP6 is a derivative of VS55 modified by Organ Recovery Systems [13].

Results and discussion

Thermal expansion of cryoprotectant solutions in cryogenic temperatures is largely unknown. For example, even if data on the thermal expansion of each component of the mixture VS55 is available, the combined effect of thermal expansion of the mixture as a whole is complex and cannot be predicted. The combined effect in cryogenic temperatures, where each component undergoes phase transition within a different temperature range, is even more complex. When VS55 is rapidly cooled, vitrification (or glass formation) is expected, where a dramatic thermal expansion is known to occur only at the glass transition temperature [1]. In practice, pure vitrification is not easy to achieve in sizable samples, and coexistence of vitrified material and crystallized material is likely to occur [2].

Thermal expansion of DMSO

DMSO solution is one of the most extensively studied cryoprotectants, and is a dominant component in each of the cryoprotectant mixtures VS55 and DP6. Figure 1 presents available data from the literature on DMSO density [10]. The linear thermal strain of DMSO in a specific process can be calculated from:

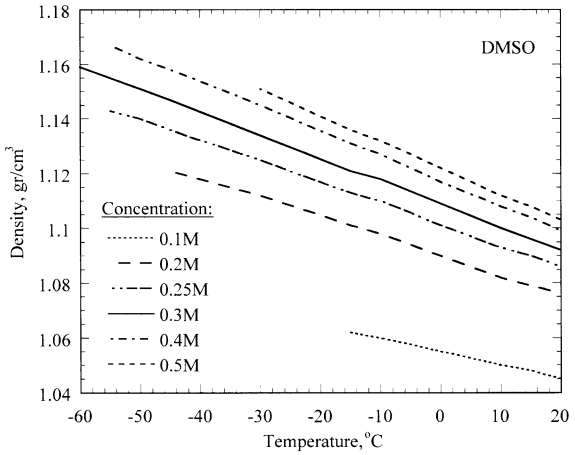

Figure 1. Density of DMSO solutions [10] (M represents mole fraction).

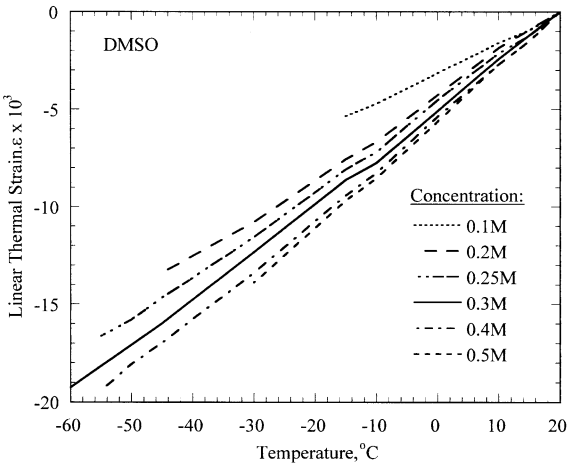

Figure 2. Linear thermal strain of DMSO solutions compiled from [10] (M represents mole fraction).

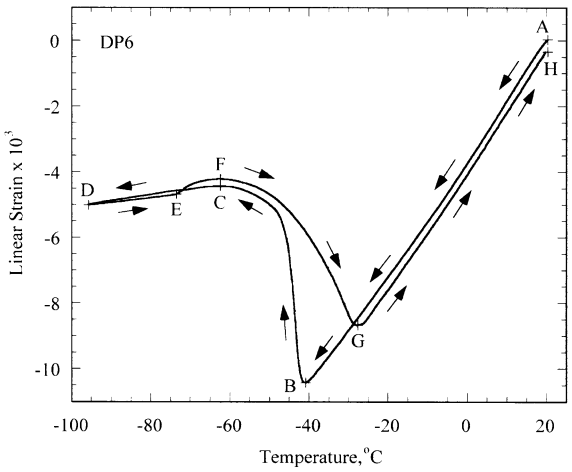

Figure 3. A typical DP6 thermal strain measurement cycle.

$$\varepsilon_i = \frac{1}{3}\frac{\left(\rho_i^{-1} - \rho_0^{-1}\right)}{\rho_0^{-1}}, \tag{1}$$

where ρ is the density, the index 0 represents a reference value at a reference temperature, and the index i represents the density at a specific temperature T_i. The thermal expansion coefficient is the rate of change of the thermal strain with respect to temperature:

$$\beta = \frac{\partial \varepsilon}{\partial T}. \tag{2}$$

Based on the data shown in Fig. 1, Fig. 2 presents the thermal strain of DMSO in a cryogenic process which starts at a standard room temperature of 20 °C. It can be seen from Fig. 2 that the thermal expansion coefficient (the slope of the curves shown in Fig. 2) can be fairly approximate as a constant value for a given DMSO concentration.

Thermal expansion measurements of DP6

Figure 3 presents a typical DP6 measurement cycle, using the new experimental system. With reference to the letter symbols in Fig. 3, the temperature-thermal strain cycle develops as follows. The cycle starts at point A, with the immersion of the cooling chamber and its thermal insulation shell into liquid nitrogen, as described in detail in Part I of this report. Segment A–B is related to sample cooling in which the cryoprotectant mixture behaves like low viscosity liquid. An almost linear relationship of the strain versus temperature is observed in this segment, which merely indicates a constant thermal expansion coefficient. Point B is found to be at a temperature of -41 ± 1 °C ($n = 6$), and is likely to be associated with a high crystallization rate of at least one component of the cryoprotectant mixture. Note that pure water starts to crystallize at 0 °C, however, as long as ice crystals occupy only a small fraction of the solution volume, and as long as ice nuclei are uniformly distributed in space, the freezing solution can be considered liquid in the solid mechanics sense. The average cooling rate in this segment is 0.85 °C/min, as can be calculated from Fig. 4. Figure 4 also shows the temperature difference between the center of the cooling chamber and the cooling chamber wall, as described in detail in Part I of this report. This temperature difference bounds the maximal temperature variation around the average temperature, at each given point in the thermal process.

Crystallization continues in segment B–C, accompanied by a dramatic decrease of thermal strain (the solution expands between points B and C). The thermal strain at point C (~−63 °C, a temperature below which 3 M DMSO is known to have high viscosity), is the same as the thermal

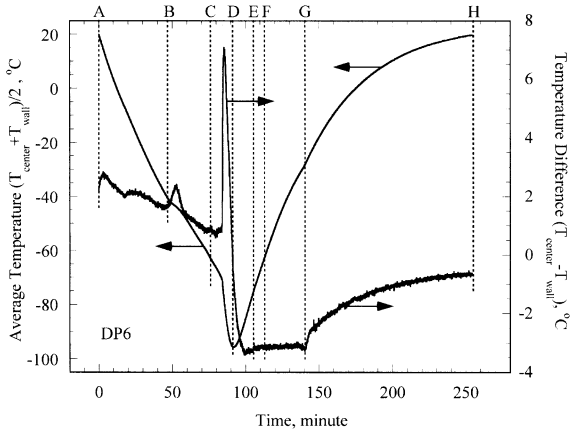

Figure 4. Thermal history in the cooling chamber during a typical DP6 cycle of strain measurements, presented in Fig. 3. T_{center} is the measured temperature at the center of the cooling chamber, and T_{wall} is the measured temperature of the outer wall surface.

strain of the mixture at a temperature of −4 °C in segment A–B. This observation can have a dramatic impact on related solid mechanics' analysis [7–9], where trapped pockets of cryoprotectant will initially contract in the liquid phase, but will have a tendency to expand at a later stage, as the cooling process progresses. When thermal expansion is constrained, mechanical stress develops.

With reference to Fig. 4, the cooling chamber is transferred from the liquid nitrogen container to free air at room temperature of about 20 °C in segment C–D. This transfer is characterized by a sudden change in temperature difference in the cooling chamber (due to cooling chamber handling), followed by a change in sign of the temperature difference (during rewarming, the wall temperature is higher than the center of the chamber).

Rewarming starts at point D, where melting is likely to be associated with segments E–F–G. Minimal thermal expansion during rewarming is observed at point F. The thermal strain curve during rewarming in segments D–E–F–G does not follow the thermal strain curve during cooling in segments B–C–D. Point G is found at a temperature of -27.5 ± 0.5 °C ($n = 6$). The observation that thawing is not the inverse process of freezing in the solid mechanics' sense, may have a dramatic effect on related solid mechanics' analyses and simulations. Comparing the maximal temperature difference in the cooling chamber (<3.5 °C) with the temperature range in segments B–C–D (55 °C) reveals that the above effects are not related to non-uniformity in temperature distribution within the cooling chamber.

Segment G–H is related to rewarming of a low viscosity liquid, where the slope of the curve (the thermal expansion coefficient) in this segment follows very closely the slope of the curve in segment A–B (cooling).

A residual strain of 3.6×10^{-4} is observed at point H, at the end of the thermal cycle. This linear thermal strain corresponds to a volumetric strain of 1.08×10^{-3}, which equals to 12 μl of solution. A similar effect has been noted in all experiments. Comparison of segments A–B with G–H suggests that this is an offset in measurement between cooling and rewarming, as further discussed below. Such an offset can be caused by change in direction of pressure measurement, in the pressure transducer. Note that 0.36×10^{-4} conforms with our estimation of uncertainty in measurement, as described in detail in Part I of this report. It is further noted that the uncertainty value discussed in Part I relates to random effects, while the offset observed in Fig. 3 is related to a systematic difference.

Analysis of thermal strain and thermal expansion coefficient of DP6, when it behaves like low viscosity liquid, is shown in Figs. 5 and 6, which are related to cooling (segment A–B in Fig. 3), and rewarming (segment G–H in Fig. 3), respectively. The technique of analysis is discussed in detail in Part I of this study. As discussed there, the absolute value of strain is not of particular interest, and only the change in thermal strain, compared to a reference value, is of significance in the solid mechanics' sense. Less than 1% difference in thermal expansion coefficient, β, is observed between cooling, Fig. 5, and rewarming, Fig. 6. For comparison purposes, the thermal strain of 3 M DMSO is also presented in Fig. 5, which is the concentration of DMSO in DP6. In broad terms, the thermal strain developed in 3 M DMSO is about 40% higher than that developed in DP6 over the same temperature range. It follows that the thermal expansion coefficient of 3 M DMSO is

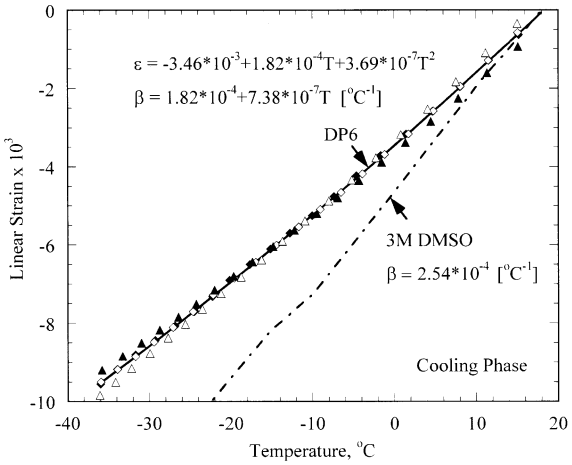

Figure 5. Thermal strain and thermal expansion coefficient of DP6 during cooling, when the cryoprotectant mixture behaves like low viscosity liquid (correspond to segment A–B in Fig. 3). Compiled thermal strain of 3 M DMSO solution [10] is presented for comparison.

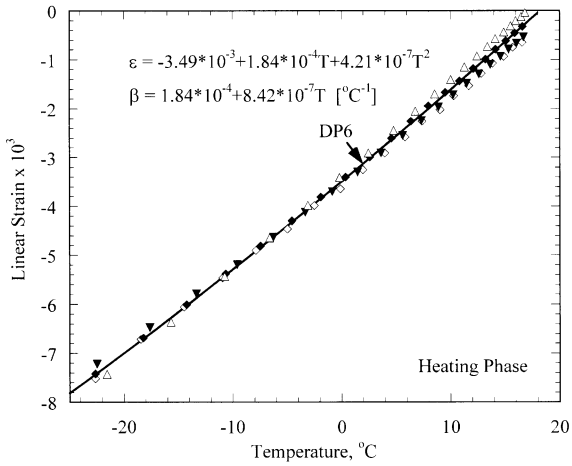

Figure 6. Thermal strain and thermal expansion coefficient of DP6 during rewarming, when the cryoprotectant mixture behaves like low viscosity liquid (correspond to segment G–H in Fig. 3).

about 40% higher than that of DP6, in the low viscosity region.

Thermal expansion measurements of VS55

A typical temperature-thermal strain curve for VS55 is presented in Fig. 7. For consistency,

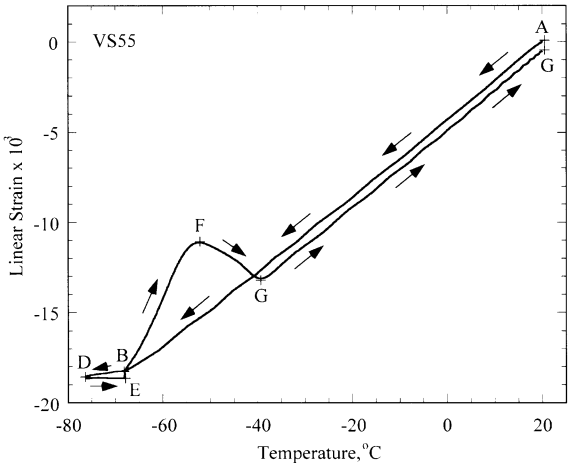

Figure 7. A typical VS55 thermal strain measurement cycle.

assignment of letter symbols in Fig. 7 is similar to that in Fig. 3 (DP6). By contrast to DP6, no volume increase effect associated with freezing is observed during VS55 cooling (at similar cooling rates). However, volume increase and subsequent decrease is observed during VS55 rewarming (segments D–E–F in Fig. 7). It is speculated that although the cooling rate was relatively low, no significant crystal formation has developed during cooling, where crystal formation is the sole effect responsible for volume increase during phase transition. It is concluded that both crystal formation (segment E–F), and subsequent melting (segment F–G), occurred during the rewarming phase. It is noted that the total concentration of cryoprotectants in the VS55 mixture is higher than in the DP6 mixture, where the major difference is the presence of 3 M formamide in VS55.

As with DP6 when the cryoprotectant behaves like low viscosity liquid, thermal expansion of VS55 is similar during cooling (segment A–B) and rewarming (segment F–G). Note that the strain difference between point A and G, and between point B and E are identical. This observation supports the previously made assumption that the residual strain at point G is a result of the mechanical characteristics of the pressure transducer, due to a change in the direction of measurements. The strain difference between point A and G corresponds to an absolute volume difference of 18 μl.

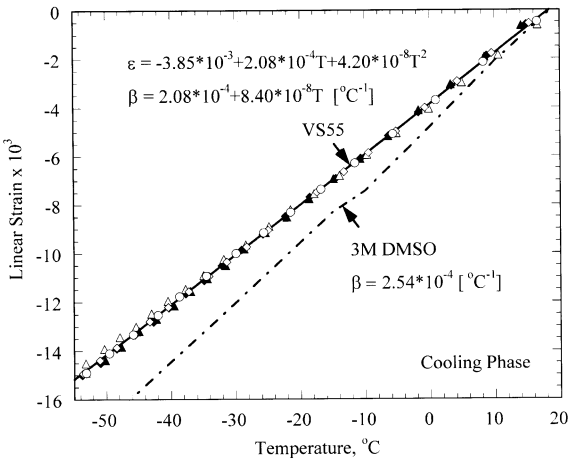

Figure 8. Thermal strain and thermal expansion coefficient of VS55 during cooling, when the cryoprotectant mixture behaves like low viscosity liquid (correspond to segment A–B in Fig. 7). Compiled thermal strain of 3 M DMSO solution [10] is presented for comparison.

Figures 8 and 9 present the thermal strain analysis and the corresponding thermal expansion coefficient for VS55, where the mixture behaves like low viscosity liquid. At this range, the thermal expansion coefficient of VS55 is 15% higher than that of DP6. The thermal expansion of 3 M DMSO is 22% higher than that of VS55. Calculations of thermal expansion coefficient, based on the cooling phase (segment A–B in Fig. 7), and on the rewarming phase (segment H–G in Fig. 7), are within 2% agreement.

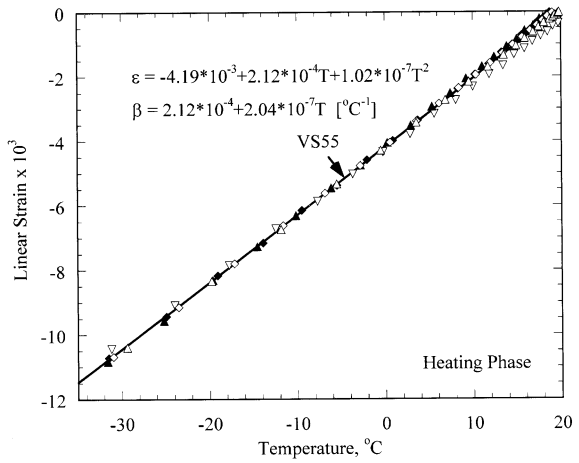

Figure 9. Thermal strain and thermal expansion coefficient of VS55 during rewarming, when the cryoprotectant mixture behaves like low viscosity liquid (correspond to segment F–G in Fig. 7).

Thermal expansion measurements in freezing solutions

The experimental setup developed for the current study is designed primarily to measure thermal expansion of incompressible fluids, where thermal strain at any given location in the cooling chamber is directly converted into a liquid level change in the pressure tube. If the sampled material contained in the cooling chamber behaves like solid, thermal strain generates mechanical stress, instead of a liquid level change in the pressure tube. It can even be argued that thermal expansion measurements during phase transition are inadequately measured in the current study. While the authors of this report do not argue otherwise, the authors further suggest the following arguments:

(i) Using the new measurement apparatus, thermal strain and its derivative, the thermal expansion coefficient can be measured at a high level of certainty when the material behaves like low viscosity liquid (segments A–B and G–H in Figs. 3 and 7).

(ii) Maximum temperature difference within the cooling chamber at any given point in time is an order of magnitude smaller than the phase transition temperature range of the studied cryoprotective solutions. It follows that phase transition effects are relatively uniform across the cooling chamber.

(iii) The thermal effects observed during phase transition qualitatively represent thermal effects in unconstrained conditions, namely volume increase upon crystal formation, and volume decrease upon crystal melting.

(iv) Volume increase, due to crystal formation in an unconstrained solution, will not be less than the volume increase measured in the current system (between point B and C in Fig. 3, and between point E and F in Fig. 7), where a portion of the thermal strain effect may have been converted into a mechanical stress. Likewise, the volume decrease upon melting in an unconstrained solution will not be less than

the volume decrease measured in the current system (between point F and G in Figs. 3 and 7).

Summary and conclusions

Thermal expansion measurements of DP6 and VS55 have been performed. When the cryoprotectant mixture behaves like low viscosity liquid, it has been shown that: (i) the thermal expansion coefficient of VS55 is 22% lower than that of 3 M DMSO, (ii) the thermal expansion coefficient of DP6 is 40% lower than that of 3 M DMSO. Since the only major difference between VS55 and DP6 is the presence of 3 M formamide in VS55, it can be concluded that the presence of Formamide increases thermal expansion.

The water portion in the VS55 mixture is lower than the water portion in the DP6 mixture, and is also lower than the water portion in 3 M DMSO. However, when the mixture behaves like low viscosity liquid, the thermal expansion coefficient of DP6 is lower than VS55 and 3 M DMSO. This observation suggests that the water has a minor effect on the overall thermal expansion coefficient when the cryoprotectant mixture behaves like low viscosity liquid.

A significant volume expansion has been observed during phase transition. While the level of certainty in thermal expansion measurements during phase transition is debatable, it is argued that it represents the lower limit for thermal expansion in an unconstrained solution.

A completely different behavior has been observed during cooling of VS55 and DP6 at low temperatures, where the cryoprotectant behaves like highly viscous material, crystallized material, or a combination of both. Future study is planned to map the role of each component of the cryoprotectant cocktail on the overall effect of volume change.

The technique presented in this study is applicable to vitrification processes, where the solution can be considered liquid for all practical cases in most of the cryogenic temperature range. Modification of the cooling chamber will enable comparable measurements at the high cooling and rewarming rates required for vitrification.

Acknowledgments

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