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Temperature characteristics of crystal storage devices in a CP100 dry shipping Dewar

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With the advent of ‘courier crystallography’, the safe and reliable transportation of cryo-cooled crystals in dry Dewars has become an important factor in the outcome of an experiment. The results of measurements of the long-term temperature characteristics of a dry Dewar are presented, together with the variation in properties of a ‘cryo-vial’, a ‘kryo-vial’ and a Rigaku MSC ACTOR ‘puck’. The short-term effects of removing and replacing the top-hat (‘cryo-cap’) holder from the Dewar have also been investigated, and the implications for best practice on synchrotron trips are discussed.

1. Introduction

The use of dry Dewars for the transportation of cryo-cooled crystals to synchrotrons is a necessity because of the safety issues associated with carriage of liquid nitrogen on aircraft. Currently, the way in which cryo-cooled crystals are stored for transport is changing because of the advent of automated sample mounting robots, which are actively being developed and installed at most synchrotron sites. Both of these issues, together with the need to keep cooled crystals at temperatures below that of the vitreous–crystalline phase change of water at 130 K (Weik *et al.*, 2001), mean that it is of interest to determine the characteristics of the combination of a dry Dewar and various crystal storage options.

Historically, the success rate with crystals in loops, stored on pins and top-hats in cryo-vials on cryo-canes (Garman & Schneider, 1997; Rodgers, 1997; Parkin & Hope, 1998), has not been as high as is desirable. This outcome is attributable to a variety of problems, such as the vial lids becoming stuck on by accumulated ice, too much manipulation of the top-hats, and cryo-vials falling off cryo-canes during transport (although this can be avoided by the use of cryo-sleeves). Alternative methods of storage may prove to be better/more reliable/easier to use than vials, but it would be advantageous for any new system to be compatible with the recently developed robots for mounting cryo-cooled crystals from their holding cassettes (Muchmore *et al.*, 2000; Ohana *et al.*, 2004). Various synchrotrons have developed in-house crystal handling robots. The commercially available mounting robots include the ACTOR system (Rikaku MSC[1]), marcsc (Mar Research[2]) and BruNo (Bruker–Nonius[3]). ACTOR uses a solid metal puck holding 12 crystals, which may be shipped in a vertically stacked rack with four other pucks in a dry CP100 Dewar (Jencons[4]). Marcsc uses the more conventional vials, while BruNo uses vials adapted to hold the temperature lower for longer by the insertion of a 9 mm deep sintered carbon absorber into the bottom of a normal vial (marketed as the ‘kryo-vial’). Since this kryo-vial is an adapted standard vial, it could be used with both the marcsc and non-robotic transfer.

The temperature characteristics of the ACTOR puck and kryo-vial systems inside a CP100 dry shipping Dewar have been measured and compared with conventional vial transport/storage with a view to improving the overall success rate. The long-term (seven days) temperature stability of each system was recorded. Our observations complement the recent experiments reported by Ridoutt *et al.* (2004), in which the temperature of the dry Dewar was monitored during a couriered journey between Hamburg and Trieste. The temperature did not rise above approximately 93 K in the course of a week. Our results on the long-term thermal stability of the CP100 dry Dewar concur with these findings.

2. Temperature characteristics of the CP100 dry Dewar

2.1. Temperature measurement

In order to monitor the temperature inside the dry Dewar, thin film platinum resistance sensors[5] were used, chosen because of their small size and ease of use. The resistance of the sensor varies linearly with temperature, and the resistance was measured using a Fluke multimeter.

The sensors were calibrated using five data points, the line of best fit being constrained to pass through the 77 K calibration point (liquid nitrogen) because of its proximity to the temperature region to be studied and the remoteness in temperature of the other calibration points (193, 267, 298 and 358 K). The calibration points of 267, 298 and 358 K were determined using a mercury-in-glass thermometer. The point at 193 K was determined using the PT 100 sensor in an ultra-low-temperature freezer.

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[1] The Woodlands, TX, USA, http://www.rigaku.com/.

[2] Hamburg, Germany, http://www.marresearch.com/.

[3] Madison, Wisconsin, USA, http://www.bruker-axs.com/.

[4] Leighton Buzzard, UK.

[5] Supplied by Farnell, PT100 sensor, part number 721-8850.

The resistance (in Ω) was found to vary linearly with temperature as expected, according to the relationship

$$T = 2.48R + 28.0\,\mathrm{K}. \qquad (1)$$

The estimated errors on the coefficients of this calibration are $2.48 \pm 0.03\,\mathrm{K}^{-1}$ and $28 \pm 1.4\,\mathrm{K}$, respectively.

So that the platinum sensors mimicked the environment experienced by a protein crystal as closely as possible, the sensors were mounted in top-hats (Oxford Cryosystems[6]), as shown in Fig. 1. The pins from the top-hats were removed so that the connecting wires could pass through the centre of the top-hat, and thus the sensor was at the crystal position.

2.2. Puck racking system

The ACTOR racking system consists of five aluminium 'pucks' (depth 5 cm), each of which is capable of holding 12 upright top-hats in separate cylindrical wells machined into the metal. The wells are 43 mm deep and are designed to hold Hampton Research[7] cryo-caps; the base of the well is magnetic and is shaped to complement the base of the cryo-cap, having a recessed ring around the edge. The rack magnetically holds the five pucks on thin horizontal plates spaced 5.1 cm apart, and the pucks are also retained laterally by a metal rod. In the experiments detailed below, the rack was only partially filled; four pucks were used and the fourth-lowest racking space was left empty. In order to make the pucks compatible with the flat-bottomed top-hats[8] used in this laboratory, an insert consisting of 4 mm of coated stainless steel was placed in the well containing the sensor. The bottom of the insert was shaped like a Hampton cryo-cap, while the top had a 1 mm deep central recess matching the diameter of an Oxford top-hat.

It was found that it was difficult to remove the pucks from the rack, particularly at cryogenic temperatures; this difficulty was due to the magnitude of the magnetic attraction between the bases of the pucks and the thin horizontal plates of the rack. In order to attenuate the magnetic flux, 1 mm of aluminium was attached to each plate on the rack. The pucks were then still magnetically retained in the rack, but they were much easier to remove. To accommodate the thicker horizontal rack plates, the gap between them was increased by adding 1 mm spacers to the rack. An alternative solution would be to replace the plates with material that is less easily magnetized. The absolute requirement for compatibility at automated beamlines with robotic mounting means that modifying or changing any robotic hardware (cryo-pin style and length, top-hats, spacers, pucks *etc.*) is inadvisable and may prevent successful use of robotic systems. In the experiments reported here automated crystal mounting robots were not used, but the robotic hardware was found to very beneficial for crystal transport and storage prior to manual mounting at the synchrotron. New cryo-tongs were designed to facilitate manipulation of top-hats into the pucks after crystal fishing. Manually removing crystals from a puck onto a goniometer or removable arc requires a single manipulation, whereas the transfer of a top-hat from a vial to an arc takes two, and into tongs three (including removing lids). Fewer manipulations generally allow less scope for experimental error.

Figure 1
Platinum sensor mounted on a top-hat. The heat-shrinkable tubing used to insulate the resistance element from the top-hat is visible in the picture. The length of the scale bar is 5 mm.

2.3. Experimental procedure

Firstly, the long-term temperature stability of the dry Dewar was monitored. The (warm) empty dry Dewar was completely filled with liquid nitrogen five times over the space of four hours. After the last fill, all the liquid nitrogen was drained from the dry Dewar. This experiment was carried out three times, firstly with canes, cryo-sleeves and normal vials, secondly with canes, cryo-sleeves and kryo-vials, and thirdly with pucks and the rack. After the dry Dewar had been cooled, the pre-cooled cane, vials/rack and pucks were placed in the Dewar and the dry Dewar lid was replaced; the time was defined as $t_1 = 0$. The temperature was then measured at regular intervals without removing the lid or moving the Dewar; these measurements were achieved by passing the temperature sensor wires out of the Dewar along a small indentation in the lid.

In order to determine whether the position within the Dewar had an effect on the temperature, each of the above experiments was carried out using two platinum sensors. One of the sensors was placed in the lowest possible racking/cane position, whilst the other was placed in the uppermost racking/cane position; this setup was repeated for each type of vial and for the pucks.

All of the experiments took place in an air conditioned room in which the temperature was kept constant at 292 K.

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[6] Long Hanborough, Oxon, UK.
[7] Laguna Niguel, CA, USA.
[8] Long Hanborough, Oxon, UK.

3. Results

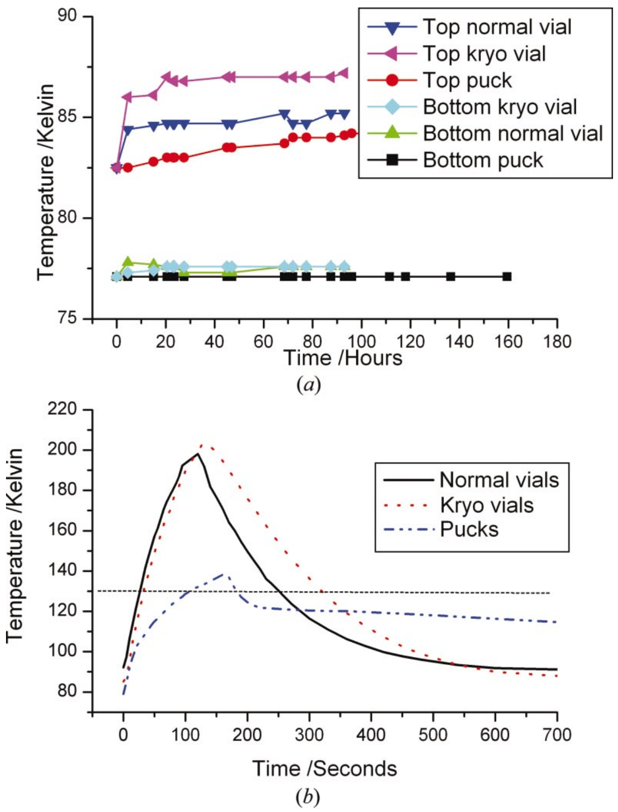
3.1. Long-term temperature variation

In order to measure long-term temperature variations in the Dewar, changes in temperature were monitored over the space of seven days. It can be seen in Fig. 2(*a*) that there is little variation in temperature within a closed dry Dewar over this time.

3.2. Short-term temperature variations

Since it had been established (§3.1) that the dry Dewar was thermally stable over long periods of time, it was clear that the most significant changes in temperature must occur when the Dewar is opened and the samples are removed (defined as $t_2 = 0$).

Thus we monitored the effect of removing the rack and vials from the Dewar for approximately 155 and 110 s, respectively, before returning them to the dry Dewar. The times were chosen with the aim of determining the time taken for the temperature to rise above that of the vitreous–crystalline phase change of water at 130 K. The resulting temperature changes are shown in Fig. 2(*b*).



(*a*)

(*b*)

Figure 2
Temperature characteristics of a CP100 dry Dewar: (*a*) shows the long-term thermal stability of the Dewar and the existence of a temperature gradient within the Dewar, and (*b*) shows the temperature change when standard vials (black continuous line), kryo-vials (red dashed line) and pucks (blue dotted line) are removed from the dry Dewar at $t_2 = 0$. The vials were returned to the Dewar after 110 s and the pucks after 155 s.

Table 1
Comparison of boil-off times.

Each time is an average of five readings and is measured in seconds.

Puck insert type			Vial type	
None	4 mm	21 mm	Original	Kryo
266 ± 34	221 ± 43	34.4 ± 3.6	28.0 ± 2.5	42.6 ± 3.0

All these measurements were taken with the vial/puck/sensor assembly being removed from a dry Dewar that had not been topped up with liquid nitrogen since the Dewar was initially cooled at the start of the long-term measurements. The purpose of this approach was to exaggerate any temperature changes and to provide data that could be viewed as a worst case scenario, since normally liquid nitrogen would be added before the crystals were removed.

Fig. 2(*b*) shows the effect that the larger thermal mass of the puck has on its temperature change upon removal from the Dewar. The time taken for the temperature to reach 130 K increases from 30 s when using either of the vials to 105 s for the pucks. When returned to the Dewar, the puck cools at a slower rate than both vials as a result of its larger thermal mass, and the standard cryo-vial cools faster than the kryo-vial.

3.3. Boil-off rates

In order to gain an idea of how much time is available for the manipulation of crystals outside of a Dewar, the times taken for nitrogen to boil below the level of the crystals were measured by eye for the following cases (Table 1): puck well as supplied, puck well with a 4 mm steel plug insert and puck well with a 21 mm plug insert. The boil-off rates for the vials in current use and the kryo-vials were also measured. These times give an indication of the time for which the crystal remains under liquid nitrogen during manipulation of the vials and puck.

4. Discussion

The dry Dewar clearly works very well, keeping crystals at the bottom of the Dewar at temperatures below 80 K and those at the top below 90 K for at least a week, agreeing with the observations of Ridoutt *et al.* (2004) who positioned their sensor half way up the Dewar.

Any crystal damage due to temperature change will occur when the rack is removed from the Dewar. It can be seen from Fig. 2(*b*) that the time taken to reach the phase transition temperature of vitreous water is fairly short (less than 100 s). This observation implies that the Dewar should always be filled with liquid nitrogen prior to the rack being removed. This approach results in the vials and the wells in the pucks being filled with liquid nitrogen, thus keeping the crystals stored within the wells colder for longer.

From Fig. 2(*a*) it can be seen that there is a temperature gradient within the Dewar; for short-term transport of crystals

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this should not adversely affect the cryo samples, as despite the temperature increasing towards the top of the Dewar, it still remains well below the critical temperature of 130 K.

After a small initial temperature increase in the case of both vial types at the start of the experiment (Fig. 2*a*), the temperature gradient remains approximately constant over an extended period of time. However, this initial temperature increase at the top of the Dewar is not reproduced at the position of the lower sensor.

The temperature gradient observed between the top and bottom of the Dewar has three possible causes. The first is that the lid is the 'weakest link' in the thermal properties of the Dewar and is the primary source of heat transfer into the interior of the Dewar. The rate of this heat transfer would increase if a dry Dewar was placed on its side during shipping, allowing heat to flow into the Dewar. However, when this possibility was explored, it was found that the temperature both at the top and at the bottom of the Dewar did not change by more than 1 K, in agreement with the observations of Ridoutt *et al.* (2004).

Secondly, the effect of the sensor cables (65 cm in length) should not be overlooked. The effect of any heat transfer through the cables will be an offset in all the temperatures measured. The cables to the bottom sensor have an extra 20 cm of their length contained within the Dewar, giving a smaller offset for the lower sensor.

The third possible reason is that, when the dry Dewar is initially cooled, the absorption material near the base of the Dewar is under liquid nitrogen for a longer time than the absorption material near the top. As the absorption material has a high specific heat capacity this difference results in the interior of the material near the top of the Dewar not being fully cooled. In the light of our observations, it seems most plausible that the temperature differential measured is due to a combination of the second and third hypotheses.

The dependence of the long-term thermal characteristics on the initial temperature illustrates one of the advantages of the pucks, which have a larger thermal mass compared with both types of vial, minimizing any temperature change before insertion into the Dewar. This aspect of the pucks is also illustrated in Fig. 2(*b*) and §3.3; the larger thermal mass of a puck means that the temperature takes longer to reach 130 K, resulting in a valuable increase in the time available for crystal manipulation. Table 1 also illustrates the importance of a careful choice of materials. The inserts were fabricated from steel in order to retain the magnetic attraction of the base of the well for the top-hat. However, unfortunately the specific heat capacity of steel is approximately half that of aluminium. Thus the introduction of a steel insert to a puck well causes a decrease in the boil-off time, both because of the poorer thermal properties and as a result of a reduction in the volume of liquid nitrogen that can boil off before the crystal is exposed.

In conclusion, of the combinations tried, the Rigaku rack and CP100 dry Dewar provide the best method for crystal transfer in terms of temperature stability. This setup is capable of keeping cooled crystals at temperatures of below 90 K for more than a week and allows time for crystal manipulation without addition of liquid nitrogen. The use of pucks has been optimized for this laboratory by the introduction of small inserts placed in the wells, to make the pucks compatible with Oxford Cryosystems top-hats, and the use of adapted tongs, allowing fewer manipulations of cooled crystals and hence less chance for ice accumulation or dropping of crystals. The pucks can also be used to hold small vials (vial height reduced from 43 to 33 mm); this setup has the additional advantage of increasing the capacity of the dry Dewar from approximately 25 samples for vials in cryo-canes to 60. This system has now been used successfully on a data-collection trip to the ESRF, where the Dewar was shipped with a combination of top-hats mounted directly in pucks and held upside down in small vials placed in the pucks.

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