An Open-Flow Cryogenic Cooler for Single-Crystal Diffraction Experiments

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Abstract

A simple, low-cost and efficient open-flow cryogenic cooling system has been constructed for crystallographic data collection from flash-cooled protein crystals on the rotation-camera station at the Stanford Synchrotron Radiation Laboratory. A constant temperature of less than 100 K at the crystal position can be maintained for an indefinite period of time. A small heater surrounding the delivery nozzle and another between the sample crystal and the sample support are used to prevent the formation of ice. The system includes an automatic filling capability so that the device can be run unattended for up to approximately one week.

Introduction

Methods for X-ray data collection from single crystals of biological macromolecules at cryogenic temperatures have been reported in the literature (Haas & Rossman, 1970; Petsko, 1975; Hope, 1985, 1988, 1990; Dewan & Tilton, 1987; Hope et al., 1989; Teng, 1990). At these temperatures, there is, in general, a dramatic improvement in crystal lifetimes and in many cases the diffraction resolution can be enhanced. The sample crystal is typically mounted at the tip of a glass fiber in a drop of inert oil (Hope, 1988), supported on an ultra-thin glass spatula (Hope et al., 1989) or on a film of cryo-protectant inside a small loop (Teng, 1990). The crystal is flash-cooled on the diffraction apparatus using the cold nitrogen stream or by immersion in a bath of liquid propane prior to mounting in the cold stream (Hartmann et al., 1982). Although there are several commercial devices that work effectively, the equipment available is relatively complex and expensive. In this paper, we describe a simple frost-free cooling device that is both inexpensive and straightforward to build. The essential feature of this design is that the nitrogen gas for the cold stream is obtained from a separate source rather than from internal boil off. The liquid-nitrogen (LN$_2$) coolant remains at atmospheric pressure and can, therefore, be replenished without affecting the flow or temperature of the gas stream. The cooler incorporates a novel heat exchanger to reduce the overall LN$_2$ consumption and can be automatically refilled during operation to provide a long-term uninterrupted supply of cold nitrogen gas at the sample position. A well insulated and robust transfer line is incorporated in the cooling device, which essentially eliminates warming of the cold-gas stream, even at reasonably low flow rates, resulting in low crystal temperature, greater temperature stability and low LN$_2$ consumption.

Description of the cooling device

A schematic diagram of the entire system is shown in Fig. 1. Numbers shown in parentheses in the text below refer to the individual components shown in Fig. 1. A 40 l Dewar (1), filled with approximately 30 l of LN$_2$, is used to cool a room-temperature nitrogen-gas stream (2) to 77 K. The Dewar, manufactured by Taylor-Wharton (Indianapolis, IN, USA), is cylindrical, with an inside diameter of 35.5 cm, and has a lid with 18 cm of styrofoam insulation (3). The gas for the cold stream is carried through the 40 l Dewar in 3/8 in (9.5 mm)-diameter soft copper refrigeration tubing (6). The tubing enters the Dewar through the Teflon tube (5) and the supplied nitrogen gas in this copper tube is, therefore, precooled a little by the escaping cold nitrogen gas. There is a two-turn circular coil (7), approximately 2 m in total length, supported ~5 cm below the lid by brackets attached to the bottom of
the lid; the coil further precools the gas stream. This arrangement consumes \( \sim 30\% \) less LN\(_2\) than one without the precooling coil. The gas is cooled to 77 K by an additional two-turn coil (8), immersed in liquid nitrogen, just above the bottom of the Dewar. A custom-built vacuum-insulated stainless-steel transfer line (9) (Cryolabs, San Luis Obispo, CA, USA), with an internal diameter of 3/8 in, is connected to the end of the submerged cooling coils using a brass Swagelok \( T \) fitting (10). The transfer line, which delivers the cold stream to the crystal position, has a 60 cm flexible bellows section for easy alignment to the sample position. The exact positioning of the cold stream is adjusted by \( X-Y \) translation stages attached to the camera apparatus. The horizontal length of the transfer line is about 80 cm. The transfer line incorporates a pump-out valve and a flange to attach it to the Dewar lid.

The temperature of the cold-gas stream is measured inside the transfer line, \( \sim 15 \) cm from the exit nozzle, using a type-\( T \) thermocouple (11) (Omega Engineering Inc., Stamford, CT, USA). The thermocouple wires exit the \( T \) fitting at the end of the transfer line through a short copper tube with a Teflon cap over the end (12). A hose clamp squeezes the Teflon-coated thermocouple wires between the Teflon cap and the copper to make a liquid-tight seal. The temperature is displayed on a three-digit LED display (13) (Omega Engineering Inc.), which also has an internal reference junction. Under typical gas-flow conditions, the temperature indicated is \( \sim 3 \) K below the actual temperature at the sample position. The inner tube of the transfer line extends about 2 cm beyond the end of the vacuum jacket. A small heating coil (14), made of 76 \( \mu \)m diameter Nichrome wire, is set in a cone of epoxy putty around this 2 cm extension.

An automatic control system (15) (Kurt J. Lesker, Clairton, PA, USA) replenishes the LN\(_2\) supply from a remote 1851 Dewar (16) (Pacific Coast Cryogenics, Saratoga, CA, USA) when \( \sim 101 \) of LN\(_2\) has been consumed, about once every 24 h. Two thermistor sensors (17 and 18), each wrapped with Teflon tape and mounted with hose clamps on a vertical wooden rod, sense the level of liquid nitrogen in the Dewar. When the LN\(_2\) falls to the level of the sensor labelled (17), the solenoid valve (19) opens and the 401 Dewar is refilled from the 1851 Dewar, through a 7 m-long insulated 1/2 in (12.7 mm)-diameter copper tube (20). This tube also enters the 401 Dewar through a Teflon-lined aperture in the lid (21). The filling continues until the LN\(_2\) level in the 401 Dewar reaches the sensor labeled (18). A third thermistor sensor (22), mounted just above the cooling coils (8), is connected to an audible alarm to warn the operator that the LN\(_2\)

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**Fig. 1.** Schematic diagram of all the components used to build the open-flow cryogenic cooler. Each individual component is numbered in the diagram and these numbers are shown in parentheses in the text.
level is low. If running continues for long after the alarm has been triggered, there will be a temperature rise at the sample position. Clearly, this alarm is only activated if either the 1851 Dewar becomes empty or there is a system failure.

Goniometer head shield
A 3.5 cm-diameter conical aluminium shield (23) prevents water condensation on the goniometer head (24) by deflecting the cold stream. It is heated by a coil of 76 μm-diameter Nichrome wire set in epoxy on its back surface. The shield can rotate freely on a stainless-steel goniometer extension post and is held in place with a circlip. Electric current for both the shield heating coil and the gas-flow-nozzle heating coil is provided by a two-channel low-voltage DC power supply (25).

Cost of components
The approximate current component price of the cooler system in the USA is $3400 (401 Dewar ~$1200; transfer line ~$1100; gas-flow meter ~$25; thermocouple ~$25; temperature readout ~$200; dual-variable DC power supply ~$700; miscellaneous parts ~$150). The price of the remote 1851 Dewar is ~$1900, and the auto-fill system costs ~$900 (electronic control unit $500; solenoid valves $150; pressure sensor $100; miscellaneous components ~$150).

Operation
Fig. 2 shows the cooler in use at the rotation-camera facility on SSRL beamline 7-1. Room-temperature boil-off nitrogen for the cold stream is supplied from a laboratory-wide system (2). The gas pressure of this laboratory-wide system is measured using an in-line pressure sensor (26). If the pressure falls below a preset threshold of ~240 kPa, an event that occurs infrequently, the two-way valve (27) in the gas supply pipe automatically switches over to obtain boil-off nitrogen from the 1851 Dewar (16). This continues until the main nitrogen-gas supply has been restored. In laboratories where there is no house supply of N₂ gas available, this gas supply can be obtained from the evaporation of LN₂ (Hope, 1990). A gas flow rate of 425 l h⁻¹ (STP; 273 K, 10⁵ Pa) is typical for a constant temperature of less than 100 K at the crystal position. When the system is operating but not being used, the flow rate can be reduced significantly (to ~301 h⁻¹) to save nitrogen. This adjustment can be made using the flow valve (28) in the gas supply line, which incorporates a gas-flow meter. The remote 1851 LN₂ tank, used to fill the 401 Dewar (heat exchanger), requires refilling about once every 9 d in practice. Nitrogen losses in the system are due to both the cooling of the delivery line (20), which is 4 m long in our case because of the location of the storage tank relative to the experimental apparatus, and evaporation within the 1851 LN₂ storage tank. If necessary, the small 401 Dewar can also be filled manually with a funnel by removing the LN₂ level-sensor rod from its Teflon feed-through hole (29) in the lid.
Fig. 3 shows a close-up view of the delivery nozzle (14) and the goniometer head shield (23) at the sample position. The cold-gas-stream direction is collinear with the sample rotation axis, which minimizes turbulence and thus reduces the chance of ice formation on the sample. It was found by trial and error that the nozzle and shield heaters prevent condensation when operated at 5 W (10 V × 0.5 A) and 7.5 W (10 V × 0.75 A), respectively, and do not have to be changed in response to changes in ambient temperature or humidity.

Results

The low-temperature system described will deliver a cold stream at constant temperature for up to 9 d without manual intervention. The lowest temperature that can be obtained at the crystal position (6 mm from the end of the transfer line), with a nitrogen-gas flow rate of 425 l h⁻¹ (STP), is ~96 K. The temperature fluctuation is less than 1 K. We have profiled the cold stream at the crystal position and the temperature variation is less than 1 K within a sphere of radius 0.5 mm.

The simple heaters completely eliminate icing and condensation and do not significantly affect the temperature at the sample position. The consumption of the liquid-nitrogen supply in the 401 Dewar is ~0.43 l h⁻¹ for a gas flow rate of 425 l h⁻¹ (STP) (the modified 401 Dewar loses ~0.13 l h⁻¹ with no gas flow). The transfer line is insulated with multiple wraps of aluminized Mylar in the evacuated space and therefore operates efficiently at a vacuum of 10 Pa or less. It has only once been necessary to re-evacuate it in the past four years of operation.

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