

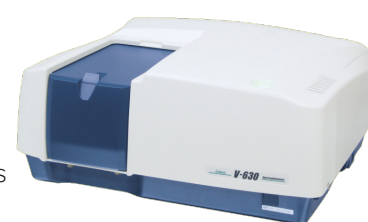
DNA Melting Measurements using the PAC-743 Peltier Cell Changer with a Temperature Sensor Inserted in the Cell

Introduction

This application note compares thermal melt measurements of a DNA sample using the temperature sensor inside a cell and using the PAC-743's holder sensor. The 8-position micro cell sensors as a temperature monitor (Figure 1), the horizontal axis of the temperature course data can be plotted with actual temperatures obtained by the sensor. This increases the temperature accuracy of small volume sample measurements with the 8-position micro cell.

For thermal melt studies, a temperature sensor can be inserted into the sample cells and the actual temperatures of samples plotted in order to increase the accuracy of the temperature readings for the melting experiment. This measurement technique is easily applied to 10 mm rectangular cells with larger sample volumes. However, for samples with small sample volumes (< 100 μL) a temperature sensor probe blocks the instrument's optical path. It is then difficult to obtain both absorbance and temperature measurements of a sample simultaneously. By using one of the cells to monitor the sample temperature without obtaining absorbance measurements at that position, the melting temperature of the sample solution can be accurately recorded.

Additionally, when performing a melting measurement on samples available in extremely small amounts, volatilization of the sample can occur at high temperatures, frequently complicating the analysis process. Placing a silicon cap on the cell can prevent sample volatilization.



V-630

UV-Vis Spectrophotometer

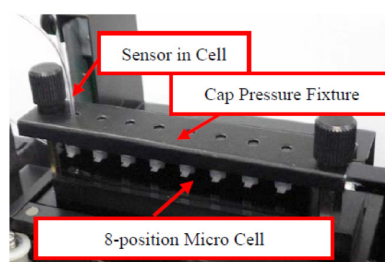


Figure 1. PAC-743 Peltier 8-position cell changer.

Keywords

V-630, UV-Visible/NIR, Biochemistry, PAC-743 water-cooled Peltier cell changer, VWTP-780 Temperature Control Measurement Program

Experimental

A 20 µg/mL solution of poly (dA-dT)-Poly (dA-dT) was made in KH₂PO₄-NaOH buffer at pH 7. Samples were measured in cells 1-7 while cell 8 was used only to monitor the sample temperature.

Measurement Conditions			
Wavelength	260 nm	Response	Fast
Ramp Rate	2°C/min	Start Condition	±0.01°C for 3 seconds
Data Interval	1°C (20-50°C), 0.1°C (50-70°C), 1°C (70-100°C),		

Results

The melting curves from the results of sample measurements with all eight micro cells using the holder sensor are plotted as shown in Figure 2. No volatilization of the samples was observed (Figure 3).

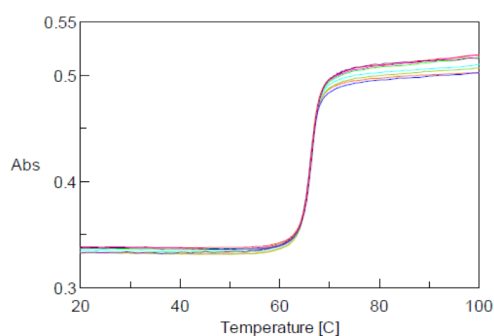


Figure 2. Thermal melt of poly (dA-dT)-Poly (dA-dT) using the PAC-743 holder sensor.

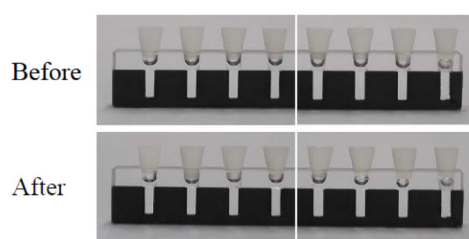


Figure 3. Change in the sample volume before and after thermal measurements.

In order to enhance the accuracy of the temperature, one of the eight cells (referred to as “cell 8”) was used exclusively to monitor the sample temperature. Figure 4 shows a result of melting curves using the temperature readings from internal cell sensor. These temperature values were plotted in the horizontal axis in Figure 4 using data collected from internal cell sensor in cell 8. No evaporation was observed in this case (Figure 5).

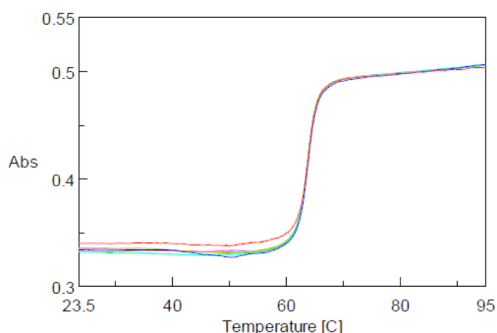


Figure 4. Thermal melt of poly (dA-dT)-Poly (dA-dT) using the internal cell sensor.

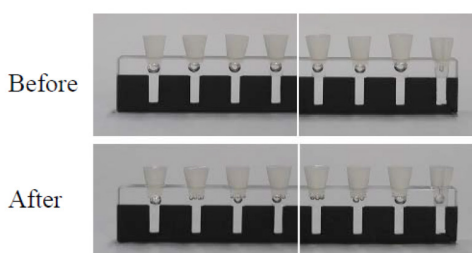


Figure 5. Change in the sample volume before and after thermal measurements.

The results of the melting points, calculated from the melting curves data in Figure 2 and 4, are shown in Table 1. The results using the holder sensor are shown in Table 1 ranging melting temperature between 66.0°C ~66.2°C (average 66.1°C) with standard deviation of 0.08°C and coefficient of variance of 0.13%. On the other hand, the melting temperature results using the internal cell sensor range between 63.6°C ~ 63.8°C (average 63.7°C) with standard deviation of 0.08°C and coefficient of variance of 0.12%.

Table 1. Recorded melting temperatures measured using the holder and internal cell sensors.

	Temperature °C	
	Holder Sensor	Internal Cell Sensor
Cell 1	66.1	63.6
Cell 2	66.0	63.6
Cell 3	66.0	63.6
Cell 4	66.1	63.6
Cell 5	66.1	63.7
Cell 6	66.0	63.7
Cell 7	66.2	63.7
Cell 8	66.2	63.7
Average	66.1	--
Standard Deviation	0.08	0.08
C.V.	0.13	0.12

Conclusions

The data show that the melting temperature using the holder sensor was approximately 3.5°C higher than using the internal cell sensor, while both the standard deviation and coefficient of variance had no major differences in their result. These results indicate that the holder sensor can sufficiently measure a reproducible melting temperature for all sample cells. However, to obtain the absolute temperature value for sample melts, a cell temperature sensor is highly recommended.